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Original Research

PROFILE OF SKIN BIOPSY PATIENTS IN DR. SOETOMO GENERAL ACADEMIC HOSPITAL, SURABAYA, INDONESIA

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ABSTRACT

Skin biopsy is an important tool used by dermatologists in diagnostic determination. The correlation between clinical and histological features is needed in understanding pathogenesis and formulating the diagnosis of a skin disease with a greatly varied spectrum of histopathological results, while the observable clinical symptoms are highly limited. Skin diseases are still a serious problem worldwide, especially in Indonesia. Based on the Indonesian Health Profile in 2010, skin diseases ranked third out of 10 most diseases in outpatients in hospitals throughout Indonesia. This study was a review of the profile of skin biopsy results in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia from 1 July 2014 to 31 July 2019, which were subjected to anatomic pathology examination. This study was an observational descriptive study using secondary data sources from the medical records at the Communication and Information Technology Installation (ICT) of Dr. Soetomo General Academic Hospital, Surabaya. Based on data searches, the total number of biopsy examinations performed was 1,368 cases. There were more female patients (50.3%) than males (49.7%). The most common skin disorder found was erythropapulosquamous disorder (30%), followed by infection (18%). Other cases consisted of skin tumor (15%), vesiculobullous (13%), connective tissue disease (7%), pigmentation disorders (5%), and vasculitis (5%). Diseases that could not be classified into 7 groups of the biopsy criteria were grouped separately in other diseases (7%).

Keywords: *Skin health; skin disease; skin histopathology; disease*

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Highlights:

1. Profile of skin biopsy results which carried out the anatomical pathology examination was reviewed.
2. The highest skin biopsy disease group case are erythropapulosquamous, infection, skin tumor, siculobullous, connective tissue disease, pigmentation disorders, and vasculitis

INTRODUCTION

Skin biopsy is an important tool used by dermatologists in diagnostic determination (James et al. 2015). The correlation between clinical and histological features is needed to understand pathogenesis and formulating the diagnosis of a disease given the spectrum of histopathological results of each skin disease that varies greatly, while clinical symptoms that can be seen are very limited. Skin disease is a serious problem in worldwide (Veldurthy et al. 2015). Based on the Indonesian Health Profile in 2010, skin diseases ranked third out of 10 highest diseases in outpatients in hospitals throughout Indonesia. This study examined the profile of patients with skin disease that underwent biopsy at Dr. Soetomo General Academic Hospital, Surabaya on July 1, 2014 to July 31, 2019 which were recorded at Hospital Information and Communication Centre.

MATERIALS AND METHODS

In this study, we used medical records of skin disease patients recorded in the ICT at Dr. Soetomo General Academic Hospital, Surabaya between which was conducted on July 1, 2014 to July 31, 2019. This study was approved by the Health Research Committee of Dr. Soetomo General Academic Hospital under a decree Number 1319/KPEK/VII/2019. Among patients applied to the hospital underwent skin biopsy and had histopathological data that were determined. Histopathological diagnoses were classified based on the criteria indicated in the textbook "Lever's Histopathology of the Skin". According to this classification, the disease groups were indicated as erytrhopapulosquamous, infectious diseases, skin tumors, vesiculobullous disease, vascular diseases, connective tissue diseases, pigmentation disorders and

other disease that consisted of metabolic disease, genodermatoses and other non-specific inflammatory disease. Demographic data of the patients and histopathological diagnoses were retrospectively evaluated. The patients were also analyzed in groups of sex and age.

RESULTS

Based on the study, skin biopsies had been obtained from 1,368 patients. The study population consisted of 688 females (50.3 %) and 680 males (49.7%) (Table 1). Age classifications consisted of children (0-14 years), youth (15-24 years), adults (25-64 years), and seniors (65 years and above). The results concluded that most cases were found in adults (25-64) with 847 cases (61.9%), while the distribution based on gender tend to vary in every group of diseases. The reasons underlying sex -based disparities in the incidence of skin and skin -related diseases remained largely unknown but were likely multifactorial. Factors that might contribute including sex difference in the structure of skin, genetic predisposition, effects of sex hormones, sociocultural behavior, environmental factors (Andersen & Davis 2017), and the difference in immune system in both genders (Klein 2012).

The group with the highest case was erythropapulosquamous with 405 cases (30%) consisting of 209 cases (51.6%) for male and 196 cases for female (48.4%). Most cases in this group were found in adults (25-64 years). An earlier study about skin biopsy also showed a similar result with papulosquamous being the most common case (Khumar et al. 2015). Costa and Bharambe (2010) had also proven that the majority of cases for erythropapulosquamous disease were found in men and the highest percentage was in the 30–40-year age group aligned with the result in Table 1.

The second group was 247 cases of infection (18%). Male was the predominant gender in this group with 161 cases (65.18%), 86 cases were found in females (34.8%), and the group of age that was mainly affected were adults with 161 cases. The result in Figure 3 presented that most cases were bacterial infections. Based on the report of the Indonesian Ministry of Health in 2012, this aligned with the fact that Indonesia still struggles with Hansen's disease or *leprosy* caused by *Mycobacterium leprae*, where the age of onsets varied but mostly appeared in the adult range of age and male predominant (James et al. 2015).

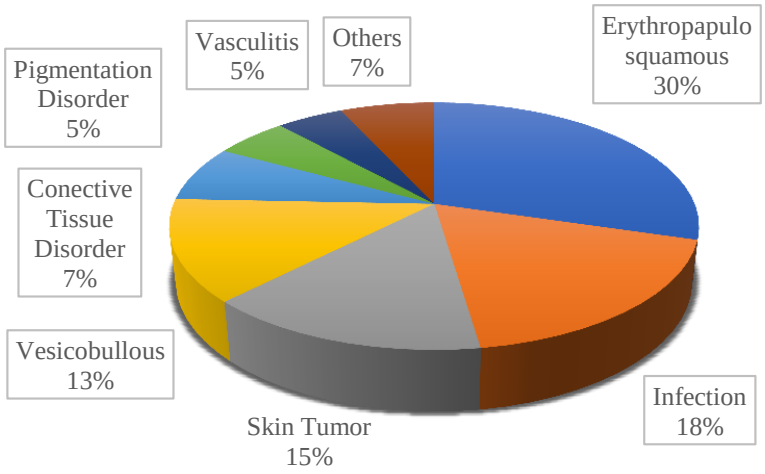

Figure 1. Results of skin biopsies

Table 1. Results of skin biopsies by age group and sex

GROUPS	Gender	Age (years)																		TOTAL
		0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	
Erythropapulosquamous	Male	6	8	10	10	12	15	15	10	15	19	27	26	16	13	4	1	1	1	209
	Female	5	8	9	7	15	13	12	13	17	26	18	22	17	8	3	3	0	0	196
	Total		46		44					281							34			405
Skin Tumor	Male	4	2	6	5	6	3	4	2	4	9	6	9	10	8	7	3	3	1	92
	Female	4	4	8	5	4	10	8	7	8	6	7	8	9	5	7	8	1	0	109
	Total		28		20					110							43			201
Vasculitis	Male	1	2	1	1	2	0	2	0	1	3	3	5	0	1	2	0	0	0	24
	Female	2	0	7	6	7	5	2	0	5	3	3	1	0	0	0	1	0	0	42
	Total		13		16					33							4			66
Connective Tissue Disorder	Male	1	2	2	1	3	1	3	1	3	5	2	0	1	1	0	0	0	0	26
	Female	1	3	6	4	6	7	5	8	10	14	4	2	1	3	0	0	0	0	74
	Total		15		14					67							4			100
Pigmentation Disorder	Male	2	1	11	4	3	5	1	1	1	2	0	0	4	0	2	0	0	0	37
	Female	2	1	7	3	2	4	3	3	2	6	1	1	1	0	0	0	0	0	36
	Total		24		12					35							2			73
Vesicobullous	Male	5	3	2	5	4	9	3	5	5	12	8	4	5	5	2	3	3	0	83
	Female	4	6	1	5	9	3	7	16	11	5	5	7	11	3	3	2	1	0	99
	Total		21		23					116							22			182
Infection	Male	3	3	5	11	18	17	12	21	22	15	10	8	5	3	4	2	2	0	161
	Female	1	5	9	5	12	7	7	8	13	2	6	5	3	1	1	0	1	0	86
	Total		26		46					161							14			247
Other Disease	Male	7	3	4	4	5	3	3	4	3	1	1	3	3	1	1	1	1	0	48
	Female	0	4	3	7	8	5	6	4	2	0	4	1	1	0	0	1	0	0	46
	Total		21		24					23							5			94
Total	Male	29	24	41	41	53	53	43	44	54	66	57	55	44	32	22	10	10	2	680
	Female	19	31	50	42	63	54	50	59	68	62	48	47	43	20	14	15	3	0	688
	Total		194		199					431							128			1368

Generally, innate and adaptive immune responses are found higher in female compared to male. These numbers are still correlated to factors from the immune system, such as females with better antibody response to viruses, men with lower $CD3^+$ and $CD4^+$ cell counts, $CD4^+$ to $CD8^+$ cell ratios, and helper T cell type 1 (Th1) responses than women, women with higher proportions of regulatory T cells and higher cytotoxic T cell activity along with up-regulated expression of antiviral and proinflammatory genes (Klein 2012). The multifactorial cause was why a female could resist varieties of bacterium, viral infections, and parasitic infestation better than male (Ahmed et al. 1985).

Skin tumor came third with 201 cases (15%), 109 (54.23%) of the cases were female, and 92 (45.77%) were male with most cases appearing in adulthood period. Based on Figure 4, we could observe that this group of diseases consisted of malignant skin tumors and other benign tumors. Malignant skin tumors consisted of melanoma and non-melanoma skin cancers, while other benign tumors consisted of *fibroadenoma*, *hemangioma*, and skin tag. Both melanoma and non-melanoma skin cancers were more frequent in male than female with a risky increase in age (Apalla et al. 2017). On the other hand, the other benign tumors dominantly consisted of female's case which included *fibroadenoma* (Greenberg et al. 1998), *hemangioma* (Glinkova et al. 2004), and a neutral gender case which included skin tag (Pandey & Sonthalia 2021).

The fourth group was vesiculobullous with 182 cases (13%). 99 cases were predominantly females (54.4%) and 83 cases in male (45.6%), while the highest number of cases were found in adults (25-64 years) with 116 cases. A cross-sectional study to evaluate vesiculobullous lesion also presented similar result with the majority of patients presented between 40-49 years old and female patients having a higher number with a male: female ratio of 1:1.27 (Arundhati et al. 2013). In accordance with the difference of immunity among genders, some of the results indicated that females had faster clearance of pathogens but also tend to have a higher susceptibility to inflammatory and autoimmune diseases (Klein 2012). Hormonal and genetic factors also contribute significantly to immune function and disease pathogenesis. Specifically, the expression of X-linked genes and microRNAs, and steroid hormones that affected responses to immunological stimuli differently in male and female (Andersen & Davis 2017). This was shown in groups with autoimmune diseases, such as *vesiculobullous* and connective tissue disease.

Connective tissue disease was ranked fifth with 100 cases (7%). Based on Figure 6, we can see that most cases in this group consisted of *lupus erythematosus* and *scleroderma* with only 2 cases of dermatomyositis. Therefore, female had a higher number of cases with 74 cases (74%) and 26 cases in male (26%). The highest occurrence for this group was also found in adults (25-64 years). In both *lupus erythematosus* and *scleroderma*, women are more frequently affected.

Scleroderma had a female-to-male ratio between 3:1 up to 14:1.1– 4 and age of onset between 30-50 years (Gottschalk et al. 2014, Odonwodo et al. 2021), while *lupus erythematosus* age of onsets varied between types, but most were included in the adult age (Goldsmith et al. 2012).

Pigmentation disorder came after with a total of 73 cases (5 %), 37 cases for male (50.6%), and 36 for female (49.3%). The age range with most cases was adults with 35 cases, but a high number of cases were also found in childhood with 24 cases. Pigmentation disorder has a diverse age of onset for each disease. Some of those in acquired pigmentation disorder were vitiligo which could appear at any age (childhood to adulthood) and had a high incidence in the second and third decade with age of onset varied between genders (Jan & Masood 2021).

Post-inflammatory hyperpigmentation also varied depending on the etiology of disease, such as acne, impetigo, dermatitis, infection, allergy, and injury (Lawrence & Aboud 2021). A congenital example is *café au lait* that were presented at birth or might even appear early in life, but the size and number of macules might increase with age (Jha & Mendez 2021).

The next group of diseases is vasculitis with a total 66 cases (5 %), consisting of 24 males (36.3%) and 42 females (63.6%). The age of distribution had increased along with age throughout childhood, youth and adulthood, but decreases in the elderly ages. One of the highest cases from this group was *Henoch Schonlein purpura* with 28 cases as seen in Figure 8. Henoch-Schönlein purpura is a vasculitis involving the small vessels that typically affects children. This disease, however, can also be seen in adults and adolescents (Robinson & Hotwagner 2021).

Diseases that cannot be classified into the 7 groups (biopsy criteria) are grouped separately in other diseases which consist of metabolic disease, *genodermatoses*, and other nonspecific inflammatory diseases with 94 cases (7 %).

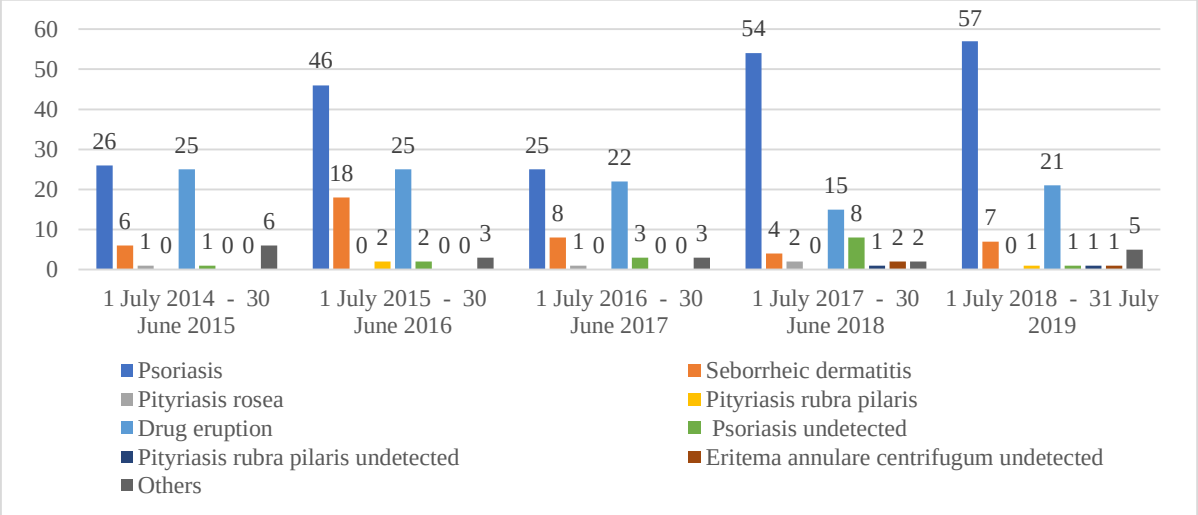

Figure 2. Results of skin biopsies of erythropapulosquamous group

Ertyhropapulosquamous is the first group with the highest number of biopsy cases. Psoriasis is the most common case with 208 cases (51.35%). Drug eruption follows with 108 cases (26.66%). Other diseases are seborrheic dermatitis with 43 cases (10.61%), pityriasis rosea 4 cases (0.98%), and pityriasis rubra pilaris 3 cases (0.74%). There were also other groups of 4.69% consisting of parapsoriasis, *lichen planus, urticaria*, and *prurigo nodularis*. Related study has shown similar result, where psoriasis dominated cases found in the biopsy of papulosquamous group of disease (Hosamane et al. 2016), thus contributing to the fact that this group was dominant in male. It could be caused by genetic predisposition and sociocultural behavior, where men had a higher chance to smoke and consume alcohol. The age group that showed the highest case were adults that might be caused by psychological stress that frequently happened in that age (Alviariza & Widyawati 2020).

Infection is the second group with the highest number of biopsy cases. The most common diseases were caused by bacterial infection with 191 cases (77.32%) that consisted of Morbus Hansen, cutaneous tuberculosis, and erythrasma followed by yeast and viral infection with 22 cases in each group. Yeast infection consists of zygomycosis, chromomycosis, deep mycoses, and chromoblastomycosis. Viral infection consisted of *verruca vulgaris* as the most

common, *verruca plan*, *condyloma acuminate*, and *molluscum contagiosum*. Next, there was also parasitic infection with 2 cases consisting of scabies and amoebiasis. Based on Figure 3, we can see that most cases were bacterial infections. The data of the Indonesian Ministry of Health in 2012 indicated that one of the most influential bacterial infections was leprosy which was still considered as a high burden in 14 provinces in Indonesia, while East Java had the highest number of leprosy patients. In adult cases, most patients were male. Although leprosy could occur at all ages, most cases appeared before the age of 35 (James et al. 2015).

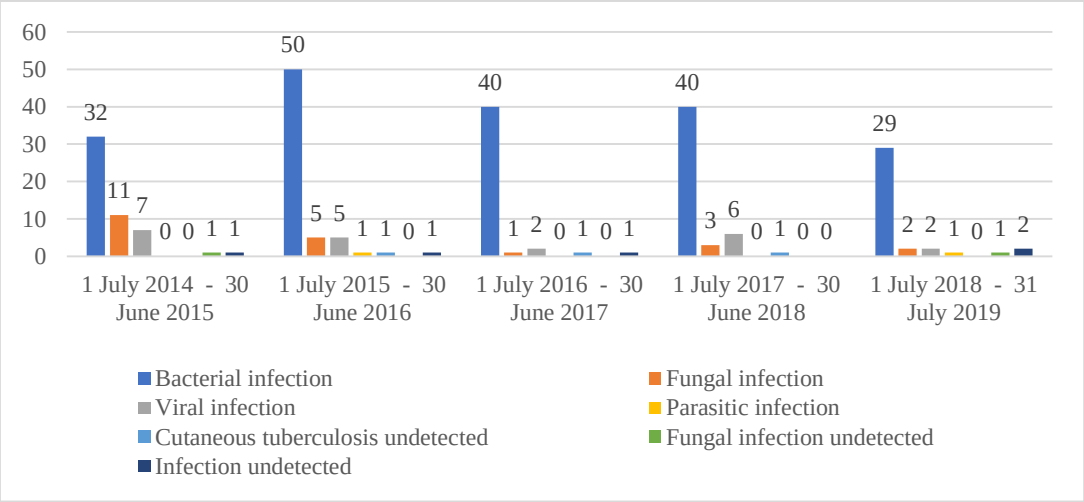

Figure 3. Results of skin biopsies of infection group

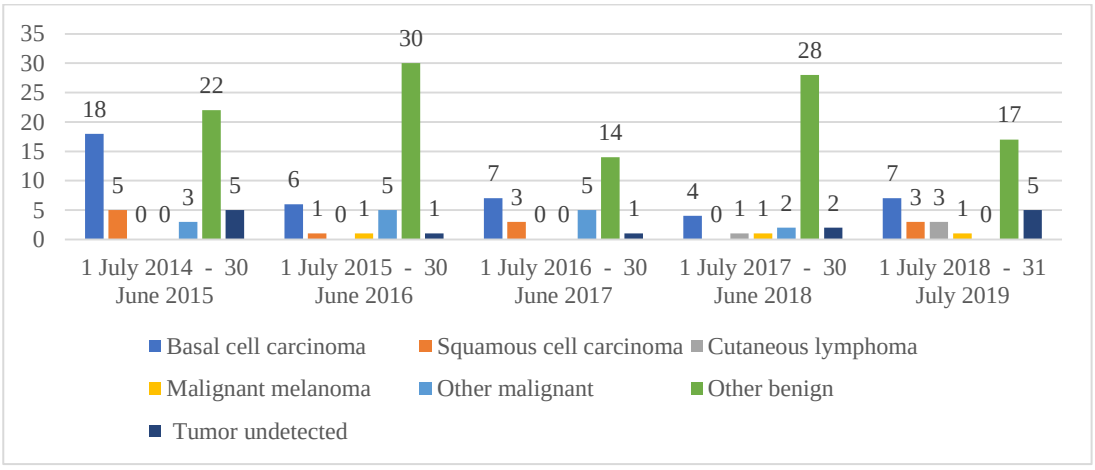

Figure 4. Results of skin biopsies of skin tumor

Skin tumor is the third group which consisted of malignant skin tumors and other benign tumors. The most common malignant tumor in this study was basal cell carcinoma with 42 cases (20.89%), followed by squamous cell carcinoma with 12 cases (5.97%). Also, there were 4 cases of cutaneous lymphoma (1.99%), 3 cases of malignant melanoma (1.49%), and 15 cases of other malignant tumors (7.46%). Apart from malignant tumors, benign tumors were also found and classified as other benign with 111 cases (55.22%) (Figure 4). The other benign tumors were fibroadenoma, hemangioma, and skin tags. Previous research also stated that the incidence of non-melanoma skin cancer was higher than melanoma. Age, gender, and genetic susceptibility are the most dominant risk factors, and the environmental risk factor was UVR exposure (Apalla et al. 2017).

Vesiculobullous disease is the fourth group with spongiotic dermatitis as the most common case with 54 cases (29.67%), followed by bullous pemphigoid with 32 cases (17.58%). The third is pemphigus vulgaris with 27 cases (14.83%). There are also 18 cases (9.89%) of pemphigus foliaceus, and 7 cases (3.44%) of *bullous impetigo*, *bullous epidermolysis*, and *dermatitis herpetiformis*. A similar result was found in an earlier study, where the most common diseases

found were pemphigus vulgaris and bullous pemphigoid (Arundhati et al. 2013) (Figure 5).

Connective tissue disease is the fifth group. The most common disease was *lupus erythematosus* with 64 cases (64%) (Figure 6). Then, *scleroderma* with 33 cases (33%) and *dermatomyositis* with 2 cases (2%). Biopsy in this group was used to determine prognosis, confirmed the diagnosis and distinguished phases of the disease (Winfield & Jaworsky 2009). Connective tissue disease is also an autoimmune-related problem that correlates with the state of sex hormones. Women undergo three major endocrinological transitions, namely puberty, pregnancy, and menopause. These endocrinological transitions exert significant effects on immune system. Another factor that contributes as a risk factor is the use of contraceptive pills (Oliver & Silman 2009) (Figure 6).

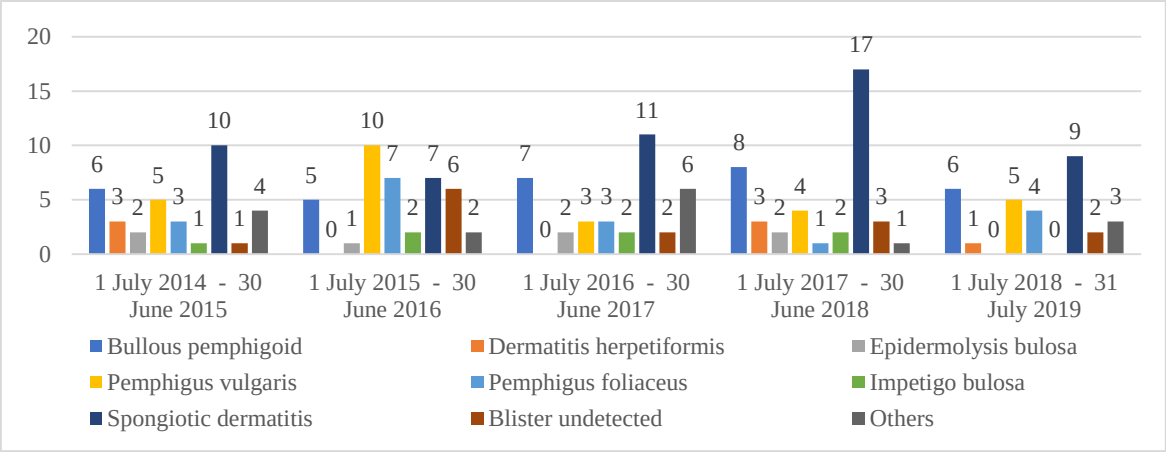

Figure 5. Results of skin biopsies of vesicobullous group

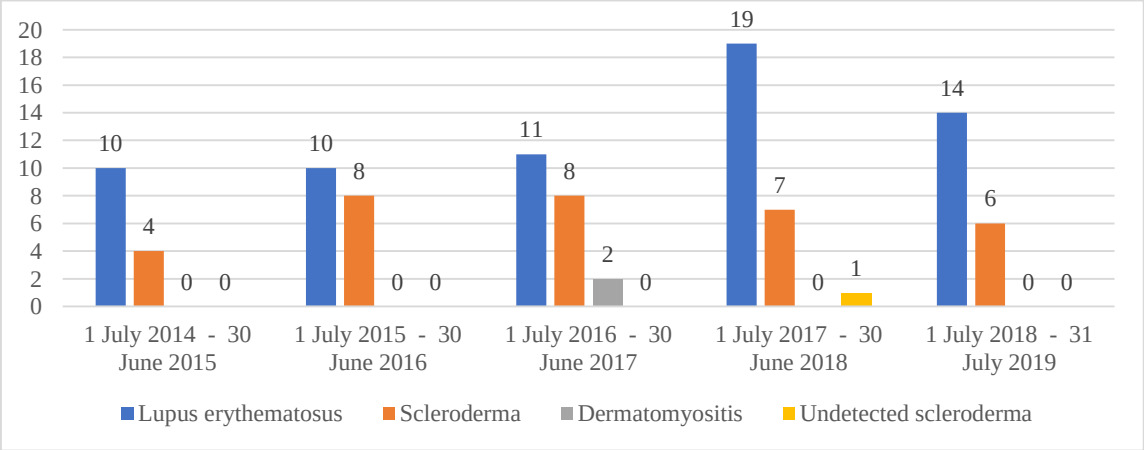

Figure 6. Results of skin biopsies of connective tissue disease

Pigmentation disorder is the sixth group consisting of congenital and acquired pigmentation problems. The most common case is nevus with 30 cases (41.09%) followed by café au lait and vitiligo with 8 cases (10.95%) each. Post-inflammatory hyperpigmentation and lentigo each were found in 6 cases (8.21%) and post-inflammatory hypopigmentation were found in 4 cases (5.47%). Biopsy in this group is often performed to differentiate benign lesions from malignant lesions. One example is malignant lentigo which is melanoma in situ on sun-damaged skin and has a similar character to solar lentigo in its initial phase, namely light brown pigmented macules (James et al. 2015) (Figure 7).

Vasculitis is the seventh group with Henoch Schonlein purpura as the highest case found with 28 cases (42.42%) followed by pyoderma gangrenosum with 4 cases (6.06%), and leukocytoclastic vasculitis with 2 cases (3.03%). An earlier study shows a slightly different result from this study where leukocytoclastic vasculitis is the highest variant of vasculitis found, followed by Henoch Schonlein purpura and urticarial vasculitis (Gupta et al. 2009) (Figure 8).

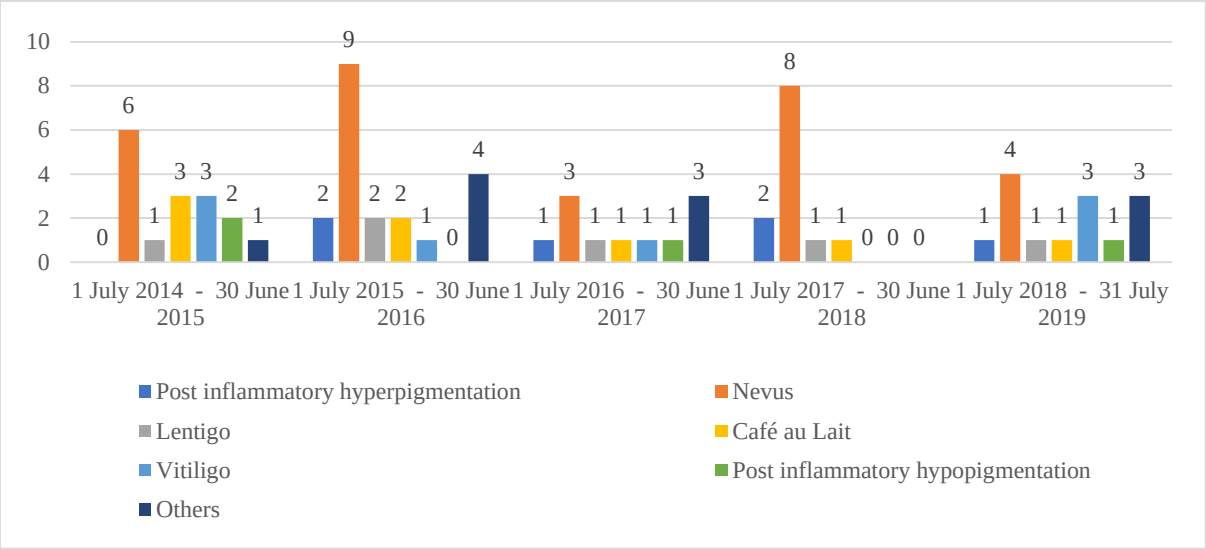

Figure 7. Results of skin biopsies pigmentation disorder

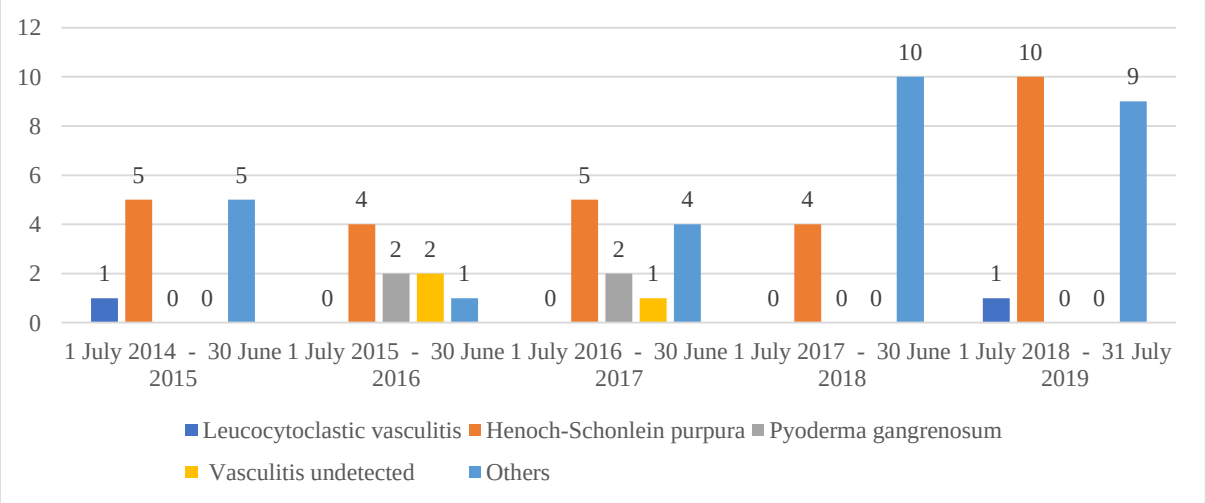

Figure 8. Results of skin biopsies of vasculitis

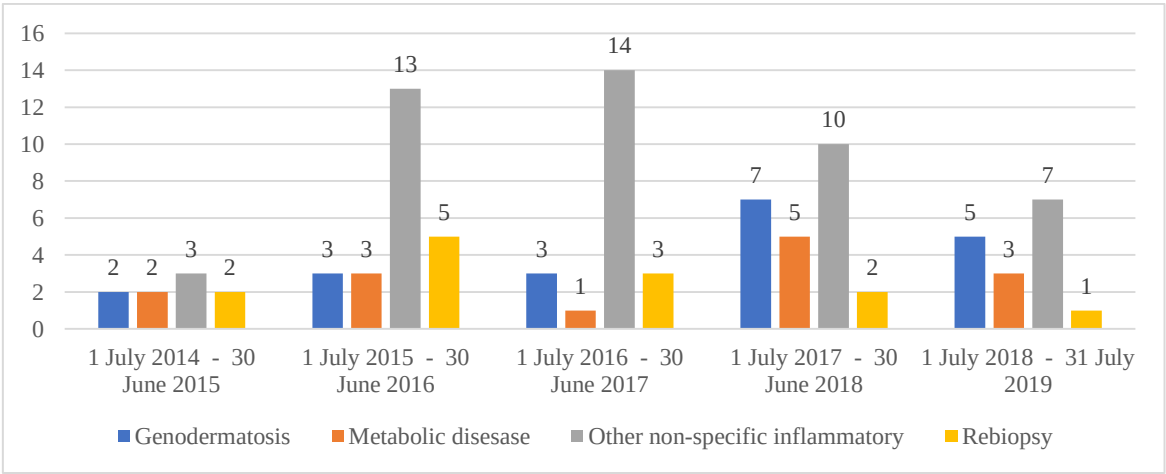

Figure 9. Results of skin biopsies of other disease

In this group, Biopsy was used mainly for diagnosis confirmation (Johnson et al. 1983). This group consisted of 48 males and 46 females, with 21-30 years old as the highest age group that underwent biopsy. This group consisted of genodermatoses with 20 cases (21.27%), 14 cases of metabolic disease (14.89%), and other nonspecific inflammatory diseases with 13 cases (13.82%). 13 cases showed the need to be rebiopsied. Genodermatoses consisted of ichthyosis and *xeroderma pigmentosum*. Metabolic disease consisted of amyloidosis (Figure 9).

Strength and limitation

The study provides insight into the profile of skin biopsy results in a large academic hospital in Indonesia, which can help improve the diagnosis and treatment of skin diseases in the country.The study covers a substantial period of five years, which can provide a more comprehensive understanding of the prevalence and distribution of skin diseases in the region. The study is based on data from a single hospital, which may not be representative of the entire population in Indonesia.

CONCLUSION

Based on the study, skin biopsies had been obtained from 1,368 patients consisting of 688 females (50.3 %) and 680 males (49.7%). Age classifications were children (0-14 years), youth (15-24 years), adults (25-64 years), and seniors (65 years and above). This study indicated that most cases were found in adults (25-64) with 847 cases (61.9%). The group with the highest case was erythropapulosquamous with 405 cases (30%) followed by infection with 247 cases (18%). Other cases consisted of skin tumor with 201 cases (15%), 182 cases of vesiculobullous group (13%), 100 cases of connective tissue disease (7%), 73 cases of pigmentation disorders (5%), and vasculitis with 66 cases (5%). Diseases that could not be classified into the 7 groups (biopsy criteria) were grouped separately in other diseases which consisted of metabolic disease, genodermatoses and other non-specific inflammatory disease with 94 cases (7%).

Acknowledgment

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Conflict of interest

None.

Funding disclosure

None.

Author contribution

All author contribution analysis data, collected data, consepsual the design study. RAAPWA was write the manuscript.

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Original Research

VIRAL LOAD AND CD4+ AMONG HIV/AIDS PATIENTS RECEIVING ANTIRETROVIRAL THERAPY IN JAYAWIJAYA DISTRICT, PAPUA PROVINCE, INDONESIA

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ABSTRACT

Highly active antiretroviral therapy (HAART) is expected to reduce human immunodeficiency virus (HIV) morbidity and mortality. Antiretroviral therapy in HIV patients is given based on clinical conditions, CD4+ cell counts, and the number of viral copies in the blood. This study aimed to determine the profile of CD4+ levels and plasma viral load in HIV patients receiving antiretroviral therapy. This was a cross-sectional study conducted within six months at Voluntary Counseling and Testing (VCT) in Jayawijaya Hospital, Papua, Indonesia. The CD4+ levels were measured using CD4+ counter and viral plasma was checked using Polymerase Chain Reaction (PCR) for 90 patients. The results showed more female patients had a CD4+ level <200 cells/mm3, a higher number of copies of the virus in the blood plasma, and stages of disease 3 and 4. Statistically, there was a significant relationship between CD4+ levels and gender with a p-value = 0.00. HIV-infected males were more likely to have lower CD4+ cell counts and higher viral loads than females.

Keywords: *Viral load; CD4+; Jayawijaya; AIDS/HIV; human immunodeficiency*

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Highlights:

1. Clinical conditions, CD4+ cell counts, and the viral copies number in the blood for AIDS/HIV were given antiretroviral therapy.
2. The profile of CD4+ levels and plasma viral load in HIV patients receiving antiretroviral therapy
3. The lower CD4+ cell counts and higher viral loads happen in HIV-infected's men.

INTRODUCTION

According to the Indonesian Ministry of Health, the HIV/AIDS case in Indonesia is increasing at the end of October–December 2017 with 14,640 HIV cases and 4,725 AIDS cases spread throughout Indonesia. Antiretroviral therapy (ART) effectively suppresses the concentration of HIV-1 RNA in the blood and reduces HIV infectivity risk (Cohen et al. 2013). Early antiretroviral therapy has been recommended as a strategy to lower HIV incidence rate, clinical evidence, and mathematical models supporting the use of ART to control HIV transmission risk at the individual level and population (Cohen et al. 2016, Eaton et al. 2012, Sorensen et al. 2012).

Although antiretroviral therapy significantly reduces the incidence of pain and mortality associated with HIV-1 virus infections, a virology treatment failure often occurs. The plasma prognostic value of HIV-1 (viral load) and the number of Cluster Differentiation 4 ($CD4^+$) for clinical progression at baseline was measured in the patient population treated using antiretroviral (Farahani et al. 2016). Therapeutic failure can be seen from a variety of criteria, virological, immunological, and clinical. The best criteria are virological criteria. If there is no examination, it uses

immunological tests. The Indonesia Ministry of Health states that ODHA should use ARV for approximately six months before the stated failure of therapy in a state of good compliance.

$CD4^+$ is the best parameter to measure immune-deficiency. If used in conjunction with a clinical assessment, $CD4^+$ can be an early indication of progression disease, because $CD4^+$ counts decline earlier than the clinical condition. $CD4^+$ monitoring can be used to initiate ARV administration or drug replacement. $CD4^+$ counts may fluctuate according to the individual and the disease suffered.

$CD4^+$ speed reduction (both absolute and $CD4^+$ percentage) has been proven to be used as a guide for the development of AIDS disease. $CD4^+$ counts decline gradually during the disease. The speed of its decline over time averages 100 cells per year (Birhan et al. 2020). The increased rate of viral load (not an absolute number of viruses) can be used to estimate the development of HIV infection. Viral load increases gradually over time. In the first three years after the seroconversion occurred, viral load changed as if only in the patient with a tendency on AIDS at the time. After that time, the change in viral load can be detected, both in the accelerometer and absolute amount. However, only both can be used as a sign progression disease.

Data from some cohort studies showed that $CD4^+$ cell counts remained steady or continued to increase in patients using ART, although there was an increase in the plasma viral load of HIV-1 in the blood. The level of immunosuppression or immunocomics determines the progression of the disease, and the $CD4^+$ count becomes a better predictor than the viral load for patients who receive ART event though predictive value of viral load that will increase with the length of infection (Shoko & Chikobvu 2019). Moreover, viral load states the measure of inhibition, while individuals with the low viral load will press the transmission rate to below (Hughes et al. 2012).

This study aimed to identify immunological clinical progression profile of $CD4^+$ levels, virology with viral load, and clinical value using clinical stage in HIV patients who have received therapy over six months in Jayawijaya Regency, Papua, Indonesia.

MATERIALS AND METHODS

This study was a descriptive-analytical study with cross-sectional design of the latitude in HIV/AIDS patients who underwent routine treatment at VCT Wamena Hospital. The research was conducted for six months from April-October 2017. $CD4^+$ and viral load were examined in 90 HIV/AIDS patients. $CD4^+$ test was conducted using a $CD4^+$-FacsPresto (BD, Bioscience, USA), while viral load was measured using the qPCR (Bioneer, Korea) technique. Sampling and recording of medical records conducted in VCT RSUD Jayawijaya, $CD4^+$, and viral-load analysis were conducted in the Laboratory of Immunology, Research and Development Center of Papua Health Office.

PCR viral load and $CD4^+$ examination, 400 ul HIV plasma extracted with viral test kit load Exiprep DX Viral RNA Extraction Kit (Cat: K-4773), and the Accupower Kit quantitative HIV PCR Kit 96 using the Machine Exiprep TM 16 (Bioneer, Korea). The results of the extraction in the elution tubes were inserted in the Vortex-spin tool (EXI-spin), and Exi-Cycler 96. Quantitative PCR results were then analyzed using Exi-CYCLER3 software. The results of the analysis were in the form of data which reflected the quantity (ml) of the virus blood samples of HIV patients.

A total of 100 ul whole blood was conducted $CD4^+$ examination using BD FacsPresto (Paint: 651000) and BD Facspresto Cartridge Kit (Cat: 655495). The $CD4^+$ test results showed an absolute value and presentation. The test result was inserted in the Excel chart for further analysis. Demographic and clinical characteristics data were obtained from patient medical records and interviews.

Quantitative PCR using Exi-Cycler could read the viral load up to >50 copies/ml of the viral RNA. The result of the <50 copies/ml entered in the category was not detected, while >50 copies/ml were detected. Data were analyzed using SPSS for $CD4^+$ frequency distribution, viral load, and clinical stage. To know the relationship of clinical progression profile and gender of patients using Chi-Square test with confidence level 0.05.

This study had been approved by the Health Research Ethics Committee, Board of Health Research and Development, Health Ministry of the Republic of Indonesia, under a decree No. LB.02.01/5.2/KE.064/ 2017.

RESULTS

Results of laboratory examination and medical record data obtained results in the form of clinical profiles of patients that include immunological, virological, and clinical parameters.

Table 1. Clinical profile of HIV/AIDS patients in RSUD Jayawijaya

Variable	Frequency	Percentage
Gender		
Male	45	50
Female	45	50
Age		
17-35years old	62	68.9
>35 years old	28	31.1
CD4+ levels		
<200 cell/mm^3	13	14.4
>200 cell/mm^3	77	85.6
Viral Load		
Detected	52	57.8
Not detected	38	42.2
Opportunistic infections		
TB	5	5.6
Non-TB	85	94.4
Type of Therapy		
EFV Based	78	86.7
NVP Based	12	13.3
Clinical Stadium		
Stage 1&2	14	15.6
Stage 3&4	76	84.4
Duration of therapy		
6-24 months	15	16.7
>24 months	75	83.3

Table 2. Relationship between progression profile and gender

Characteristics	Gender		p-value	Odds ratio	95% CI interval	
	Female	Male			Lower	Upper
CD4+ levels						
<200 cell/mm^3	12	1	0.00*	16.00	1.98	129.27
>200 cell/mm^3	33	44				
Viral Load						
Detected	27	25	0.83	1.20	0.51	2.77
Not detected	18	20				
Opportunistic infections						
TB	1	4	0.36	0.23	0.02	2.17
Non-TB	44	41				
Clinical stadium						
Stadium 1&2	5	9	0.38	0.50	0.15	1.63
Stadium 3&4	40	36				

Table 1 showed that patients with a range of 17-35 age-years who were getting antiretroviral therapy >24 months were more dominated by CD4+ levels >200 cells/mm^3, and more viral loads were still detected in the range of Stage 3 and stage 4. The viral load number was highly detected in 2 patients, i.e., 2.07 x 106 and 2.38 x 106 copies/ml, and the lowest viral load ranged from 11 copies/ml and 15 copies/ml of blood. CD4+ levels <200 cells/mm^3 the lowest was 15 cells/mm^3.

From the Table, the composition of male and female patients was balanced, so that the analysis of the relationship to identify the relationship between the gender and characteristics progressivity of the patient's disease could be obtained (Table 2).

Table 2 illustrated a meaningful relationship between gender and profile progression, and the patient was a CD4+ level with p: 0.00 (<0.05). This suggested that female patients were more at risk of having lower CD4+ values (<200 cells/mm^3) than male patients.

DISCUSSION

Gender contributes to the pathogenesis of diseases of various infectious diseases, including HIV (vom Steeg & Klein 2016). Most studies suggested that females had a lower viral load of HIV at infection onset, but despite these differences, progression diseases were comparable between genders (İnkaya et al. 2019). Females had a higher CD8 T cell activation at certain HIV viremia levels (Kovacs et al. 2010), whereas males had one log10 higher viral load activation (Meier et al. 2009). Similarly, the gene expression was stimulated to higher interference in females when controlling the viral load of HIV (Chang et al. 2013). Given immune activation's role in encouraging the progression of HIV disease and in the comorbidity that arose during the effective use of ART, the gender differences in immune set points had clinical consequences (Schwartzman-Morris & Putterman 2012).

This study showed that female HIV patients were more likely to have lower CD4+ levels than male patients. It was proven that progression disease was faster in females. Previous studies showed that disease progression in females was faster than in men. It was attributed to females who were less likely to start an ART or receive treatment for opportunistic infections and were more susceptible to suffering from anemia at the period of initiation of ART (Maskew et al. 2013).

Some contributing factors to gender differences and manifestations of the disease were the risk factors of behavior and epidemiological, socio-economic, differential expression of genes, and levels of sex steroid hormones (Ruel et al. 2011). The mechanisms underlying gender differences and manifestations of the disease HIV-1 had been investigated in In vitro. The results showed that plasmacytoid dendritic cells (pDCs) of females were more significant producing interferon-alpha (IFN-α) in response to HIV-1 derived from Logan Toll-Like Receptor (TLR) compared to plasmacytoid dendritic cells from males (Meier et al. 2009, Wang et al. 2012). Nevertheless, molecular mechanisms underlying sexual dimorphism in immune function were still researched.

Previous attempts to explain sex differences and HIV infections were focused on the various effects of the primary female sex steroid immunomodulation (Ruel et

al. 2011, Ziegler & Altfeld 2016), particularly on hormone estrogen and progesterone. Receptors for estrogen and progesterone were expressed by most types of immune cells, and these hormone levels affected the expression of CCR5, $CD4^+$ T cells, and the production of several cytokines (Mo et al. 2005, Biswas et al. 2022). This exogenous hormone was natural and fluctuated during the ovulation cycle and modulated the innate and adaptive immune response, so that it could affect the HIV replication rate (Hel et al. 2010). The viral load value illustrated the disease progression and risk of death. Periodical checking concerning the number of CD4+ and viral loads could determine the progress of the disease and identified the exact requirements to start or change the antiretroviral regimen.

Strength and limitation

The strength of this study is that it provides important information on the profile of CD4+ levels and plasma viral load in HIV patients receiving antiretroviral therapy in a specific setting in Indonesia. The study findings may be useful for healthcare providers in improving HIV treatment and management in the study population. Limitations of this study include its cross-sectional design, which only provides a snapshot of the CD4+ levels and plasma viral load in HIV patients at a specific time point.

CONCLUSION

The clinical progression of HIV-1 patients in the Jayawijaya Regency, Papua, Indonesia, showed that there were more females with HIV who had a $CD4^+$ level of <200 cells/mm^3, higher or still detectable number of copies of the virus content per ml, and with the stage of the disease of stage 3 or 4.

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Conflict of interest

None.

Funding disclosure

None.

Author contribution

DAP,MW,MIF,SA and EIN were conseptual design and collected and analysis data. MW write the manuscript.

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Original Research

PROFILE OF TUBERCULOSIS IN CHILDREN IN TAMAN DISTRICT, SIDOARJO REGENCY, INDONESIA

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ABSTRACT

The prevalence of tuberculosis (TB) in Indonesia was 391 per 100,000 population with 110,000 of deaths in 2016. This study was a descriptive study research aimed to determine the profile of pediatric TB patients using medical records at Taman public health center, Sidoarjo, Indonesia, in the period of 2016-2019. The samples in this study were 31 pediatric patients aged 0-14 years with a history of TB obtained by total sampling technique.There were 31 patients consisted of those aged >5 years (58.1%) and ≤5 years (41.9%), 83.9% of those had pulmonary tuberculosis (83.9%), and 16.1% with extrapulmonary tuberculosis. The patients aged ≤5 years had good nutritional status (32%) and those who had poor nutritional status were 27.2%. Meanwhile, those aged >5 years had poor nutritional status (22.7%) and those who had good nutritional status were 18.1%. The patients who had a history of contact with adult TB patients were 86.7% and those who did not have a history of contact with adult TB patients were 13.3%, while those who had received BCG immunization were 86.2%, and 13.8% had not received BCG immunization. This study concluded that most pediatric TB patients were >5 years old, and almost all pediatric TB patients had pulmonary tuberculosis. Pediatric TB patients aged ≤5 years were more likely to have good nutrition. Meanwhile, there were more pediatric TB patients aged >5 years who had poor nutritional status. Almost all pediatric TB patients had a history of contact with adult TB patients and had received BCG immunization.

Keywords: *Tuberculosis; tuberculosis in children; tuberculosis profile; pediatric*

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Highlights:

1. Pediatric tuberculosis patients profile using the medical records was determined.
2. The most pediatric tuberculosis patients under five years have pulmonary tuberculosis.
3. Pediatric tuberculosis patients received BCG immunization had a contact with adult tuberculosis patients histories.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *bacterium Mycobacterium* tuberculosis and currently still becomes health problem throughout the world. In 2016, there were more than 10.4 million cases of tuberculosis with more than 1.3 million people died from tuberculosis. Tuberculosis is a great problem for public health in developing countries, one of which is Indonesia. In 2016, Indonesia was ranked second in the world with around 1,020,000 cases of tuberculosis, while 60,000 cases occurred in children aged 0-14 years.

The transmission of tuberculosis in children still happens. It becomes a concern and success indicator in TB control in the community (Winston & Menzies 2012). Taman public health center has many cases of tuberculosis based on Sidoarjo Regency health profile data report. In 2014, in the working area of the Taman public health centers, there were 61 new AFB-positive cases with a total of 121 TB cases (Sidoarjo Health Office 2015). According to data in 2014, 54 cases of TB in children were reported in several public health centers in Sidoarjo. Taman public health center had the highest number of TB cases for children compared to other health center with only 15 cases (Sidoarjo Health Office 2015). Children who are exposed to *bacterium Mycobacterium* tuberculosis will be at risk of developing tuberculosis infection.

The nutritional status of children is very influential on body's immunity. Children who are malnourished will result in a decreased immune system, so that they are susceptible to tuberculosis infection (Febrian 2015). On the other hand, infectious diseases with any severity can be detrimental to any nutritional state (Fatimah et al. 2010). The infection that occurs is also influenced by several factors, including intensity and contact with adult tuberculosis patients, as well as the body's immune response (Seddon et al. 2013). Giving BCG immunization can provide immune protection in infants against TB disease. Tuberculosis in children can cause developmental disorders, even death. This study aimed to determine the profile of tuberculosis in children at the Taman public health center as an initial stage in diagnosing variety of symptoms of tuberculosis in children.

MATERIALS AND METHODS

This study used descriptive method to find the elements, characteristics, properties of a phenomenon. Starting with the data collection process, analyzing the data, and interpret (Suryana 2010). The procedure used in this study was documentation study. The instrument used in this study was medical record data for pediatric TB patients for 2016-2019 period at Taman public health center. 31 pediatric patients aged 0-14 years with history of pulmonary and extrapulmonary TB treated at the Taman public health center were the samples in this study. This study used a total sampling technique by taking into account the existing inclusion criteria. The inclusion criteria in this study were children aged 0-14 years who suffered or had a history of pulmonary or extrapulmonary TB and lived in Taman district, Sidoarjo regency.

The variables were age, type of tuberculosis, nutritional status, history of contact with adult TB patients, and BCG immunization status. Assessment of nutritional status for children aged ≤5 years was calculated using weight-for-age parameter, then interpreted based on the anthropometric standard of the child according to the Z-score of the WHO Child Growth Standards. Meanwhile, the assessment of nutritional status for children aged >5 years was calculated using BMI-for-age parameters, then interpreted based on CDC Growth Charts 2000. The data were calculated using univariate analysis in the form of percentages and presented in Table.

RESULTS

The identification of the characteristics of pediatric TB patients aged 0-14 years in the working area of Taman public health center is shown in the Table 1.

The total of data obtained from medical record data for pediatric TB patients in the period 2016-2019 were 31 data. Table 1 and Table 2 describe the characteristics of pediatric tuberculosis patients. The table explained that most of the subjects were male (51.6%). Most of the pediatric TB patients were children older than 5 years (58.1%). Almost all patients suffered from pulmonary tuberculosis (83.9%). Almost all pediatric tuberculosis patients had a history of contact with adult TB patients (86.7%). From the results of the study, almost all pediatric TB patients had received BCG immunization (86.2%).

The assessment of nutritional status for children aged ≤5 years old was calculated using the weight-for-age parameter and interpreted based on Child Anthropometric Standards according to the WHO Child Growth Standards Z-score. The assessment of nutritional status for children aged >5 years was calculated using BMI-for-age parameters, and interpreted based on 2000 CDC Growth Charts. From Table 2, most patients under 5 years old had normal nutritional status (32%). Meanwhile, most of the patients aged over 5 years old had poor nutritional status (22.7%).

Table 1. Distribution and frequency of TB in children by gender, age, classification of TB, history of contact with adult TB patients, BCG immunization status

Characteristics	Frequency (n)	Percentage (%)
Gender		
Male	16	51.6
Female	15	48.4
Age		
≤5 years old	13	41.9
>5 years old	18	58.1
TB Classification		
Pulmonary Tuberculosis	26	83.9
Extrapulmonary Tuberculosis	5	16.1
History of contact with adult TB patient	30	96.8
Known	26	86.7
There is contact history	4	13.3
There is not contact history	1	3.2
Unknown		
BCG Immunization Status		
Known	29	93.5
Immunized	25	86.2
Not yet immunized	4	13.8
Unknown	2	6.5

Table 2. Distribution and frequency of TB in children by nutritional status

Characteristics	Nutritional Status							
Known	Frequency (n)					Percentage (%)		
	22					71		
≤5 years old (Weight-for-Age)	Very low weight		Less weight		Normal		Risk of overweight	
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
	3	13,6	3	13,6	7	32,0	0	0,0
>5 years old (BMI-for-Age)	Less weight		Normal		Overweight		Obesity	
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
	5	22,7	3	13,6	1	4,5	0	0,0
Unknown	Frequency (n)					Percentage (%)		
	9					29		

DISCUSSION

According to World Health Organization (2017a), males have a higher risk of being infected and dying of tuberculosis than females. There are more than five hundred thousand boys aged 0-14 years in the world infected with tuberculosis (WHO 2017a). In Indonesia, the incidence of tuberculosis in male aged 0-14 years is reported to be more than thirty thousand cases (WHO 2017b).

The results of this study stated that most of the pediatric TB patients in Taman public health center working area were male (51.6%). Another study conducted in Yogyakarta also showed that the number of cases of pulmonary TB in males was more than females, namely 57.6% in the case group (Upe 2015). Meanwhile, another study conducted in the United States showed that pediatric TB patients were more common in females (54%) than males (46%) (Pang et al. 2014). This is in line with the research conducted by Simbolon, which showed that there was no difference in risk of pulmonary TB by gender (Simbolon 2007).

In this study, children aged >5 years old had a higher percentage than 5 years old. The distribution of pediatric tuberculosis cases by age in Taman public health center was 58.1% with age ranging from 5-14 years old. Contrary, a study had found that pediatric tuberculosis was more common in children <5 years old (Marais & Schaaf 2014). Children who are exposed to bacterium Mycobacterium tuberculosis will be risky of developing tuberculosis infection. Children aged ≤5 years are vulnerable group to health and nutrition problems. Of course, this is related to the development of immature immune system (Oktaviani 2011).

However, a study indicated that at any age, our body can fight infection only if the nutritional status is adequate (Apriliasari et al. 2018). Similarly, a study also indicated that the distribution of tuberculosis cases

in children aged 5-14 years was 62.5% (Nurwitasari & Wahyuni 2015). Another study conducted in Aceh also showed that the majority of pulmonary TB patients belonged to the productive age group (70%) (Hadifah et al. 2017). This happened, because he had been infected with bacterium Mycobacterium Tuberculosis for a long time, but it did not immediately become a disease. Bacteria will form colonies whose growth is limited by cellular immunity. These bacteria will still live in dormant form. When the condition of immune system decreases, bacteria will be active again and develop into TB disease (Werdhani 2002).

In this study, almost all pediatric TB patients had pulmonary TB (83.9%). The results of a study conducted in the United States showed that the incidence of pulmonary TB was more than extrapulmonary TB (Winston & Menzies 2012). Other studies that supported the results of this study also showed that pulmonary TB disease (68%) was more common than extrapulmonary TB (32%) (Pang et al. 2014). Meanwhile, the incidence of pulmonary TB in Spain was 126 cases out of a total of 134 patients (Soriano-Arandes et al. 2019).

Pulmonary tuberculosis is the most common clinical manifestation compared to extrapulmonary tuberculosis. This happens, because the lungs are the entry point for TB bacteria in more than 98% of TB infections. Due to its small size, it is easier for TB bacteria in infectious droplets to inhale and enter the alveoli (Werdhani 2002). In addition, the nature of TB bacteria that have an affinity for oxygen makes the lungs as a favorite organ for TB bacteria (Zombini et al. 2013).

In this study, the data obtained from the medical records of pediatric TB patients in the Tuberculosis Polyclinic at Taman public health center were incomplete, so that there were some data on pediatric TB patients whose nutritional status could not be calculated. The total number of data that could be calculated for nutritional status were 22 children, while those which could not be calculated were 9 children. From the total amount of data for nutritional status, 13 children were ≤5 years old and 9 other children were >5 years old. Nutritional status was calculated from weight-for-age for children aged ≤5 and calculated from BMI-for-age for children aged >5. There were more pediatric TB patients aged ≤5 who had normal/ good nutritional status (32%) than those with poor nutritional status (27.2%). Meanwhile, pediatric TB patients aged >5 had more poor nutritional status (22.7%) than those who had normal/ good nutritional status (18.1%).

It was found that the number of pediatric TB patients is more prevalent in children aged >5 years, because in this age group, more children had poor nutritional status than normal/ good nutritional status. This is in line with the results of other studies conducted in the region Garuda public health center Bandung city in 2013 showed the number of respondents with poor nutritional status was 13 children (59.1%) of the total number of respondents (Febrian 2015). Another study also showed that 57.5% of the case group were children with poor nutritional status (Yustikarini & Sidhartani 2015). The results of another study conducted in Jember showed the number of children in the case group with poor nutritional status was 79.2% (Nurwitasari & Wahyuni 2015).

Nutritional status is a condition caused by a balance between intake and nutritional needs needed for various biological processes of the body (Fuadiyah 2009). An imbalance between intake and the nutritional needs of the body can reduce nutritional status (Oktaviani 2011). Poor nutritional status will increase the risk of TB disease. Likewise, the disease course of TB that affects immune system will also lead to poor nutritional status (Nandariesta et al. 2019). When a child has an active disease, the inflammatory response generated will increase the rate of metabolism and cause anabolic blocks that affect absorption, distribution, and excretion of nutrients in the body, which will lead to malnutrition. Malnutrition is often associated with an increased risk of respiratory tract infections (Jaganath & Mupere 2012).

Based on the results of this study conducted at the Tuberculosis Polyclinic at Taman public health center, from 30 data with known contact history, almost all pediatric TB patients had a history of contact with adult TB patients, which amounted to 26 children (86.7%), while 4 other children (13.3%) had no history of contact with adult TB patients. Another study showed 28 respondents (70%) from the case group had a history of contact with smear-positive adult TB patients (Yustikarini & Sidhartani 2015). Another study that supported the results of this study was a study conducted in the United States, that as many as 79 pediatric TB patients (53%) had a source of transmission and 51 sources of transmission of which (65%) were close household contacts (Pang et al. 2014).

Contact history is an important indicator of scoring system used in the TB diagnosis process in children. Family is the closest contact with pulmonary TB patients. If the number of family members is large enough, the risk of transmission to vulnerable groups (toddlers) will also increase (Hadifah et al. 2017). The source of transmission of TB infection in children comes from the closest family members, which are parents or caregivers who live together for a long time (Nurwitasari & Wahyuni 2015). Children are very easy

to catch tuberculosis bacteria from adults. Adult TB patients can spread bacteria in the form of tiny infectious droplets that come out when talking, coughing, or sneezing. Infectious droplets that survive in the air can be inhaled by people around, including children (Nandariesta et al. 2019).

Children who have a history of contact with adult pulmonary TB patients have a 3.1 times greater risk of being infected with pulmonary TB compared to children who do not have a history of contact with adult pulmonary TB patients (Apriliasari et al. 2018). Sources of TB disease transmission are pulmonary TB patients with positive smear and TB transmission to children depends on the level of transmission, duration of exposure, and the child's immune system. TB patients with negative smear results can still transmit TB disease. In the 2014 National Guideline for Tuberculosis Control, it is stated that the transmission rate of positive smear TB patients is 65%, negative smear TB patients with positive culture results are 26%, while TB patients with negative culture results and positive chest X-ray are 17% (Ministry of Health 2014).

In this study, there were a total of 29 pediatric TB patients whose BCG immunization status was known from the patient's medical record data. It was found that more children had received BCG immunization than children who had not received BCG immunization, as many as 25 respondents (86.2%) had received BCG immunization, while 4 respondents (13.8%) had not received BCG immunization. In another study, 32 respondents (80%) in the case group had received BCG immunization, so that there was no relationship between BCG immunization status and TB disease in children (Yustikarini & Sidhartani 2015). Other studies that support this study also show that most pediatric TB patients have received BCG immunization, namely 66.7% in the case group and 91.7% in the control group (Afifah 2019).

In this study, almost all respondents who had received BCG immunization were still exposed to tuberculosis. This might be due to other factors that could affect the effectiveness of the BCG vaccine given (Febrian 2015). To fight TB bacteria, it is necessary to have good cooperation from the complex immune response (innate and adaptive immunity) in the body. In newborns who still do not have a perfect immune system, only rely on the response of the innate immune system and maternal antibodies in case of infection (Jaganath & Mupere 2012). The provision of BCG immunization can increase the coverage of exclusive breastfeeding against TB infection, because it can improve the child's immune system. Giving BCG immunization can affect the incidence of TB in children, because if they have not received BCG immunization in children at infancy, the child's immune system will be disturbed, so that they are susceptible to TB disease (Afifah 2019).

In several published studies, the effectiveness of BCG immunization ranges from 0% to 80%. This could be due to the differences in the type of BCG used, the differences in TB bacteria strains in different regions, differences in levels of exposure and immunity to environmental mycobacteria, and differences in immunization practices (Upe 2015). BCG vaccine cannot guarantee full protection against possible TB infection, about 68.6% who have been immunized with BCG are infected with TB. Although the immunity that is formed does not guarantee that a person is not infected with TB bacteria, BCG immunization can protect against more severe types of tuberculosis, such as miliary TB and meningitis in children, so that if a person has a TB infection, it is not progressive and does not cause severe complications (Yustikarini & Sidhartani 2015).

Strength and limitation

This study provides valuable information about the profile of pediatric TB patients in a specific location in Indonesia, including their age, nutritional status, type of TB, history of contact with adult TB patients, and BCG immunization status. The use of medical records as a data source allowed for a relatively large sample size and the use of a total sampling technique increased the representativeness of the sample. The study was conducted at a single public health center in Indonesia, so the findings may not be generalizable to other locations or populations.

CONCLUSION

Most pediatric TB patients were >5 years old, and almost all pediatric TB patients had pulmonary tuberculosis. Pediatric TB patients aged ≤5 years were more likely to have good nutrition. Meanwhile, there were more pediatric TB patients aged >5 years who had poor nutritional status. Almost all pediatric TB patients had a history of contact with adult TB patients and had received BCG immunization.

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Conflict of interest

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Author contribution

RDO, PL, and DM contributed analysis data and conseptual study. RDO was write and revised the manuscript. RAS was final check.

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Original Research

SKILL IMPROVEMENT FOR PUBLIC HEALTH CENTER STAFF IN THE MANAGEMENT OF TOXIC AND HAZARDOUS MATERIALS

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ABSTRACT

The generation of medical toxic and hazardous material (THM) waste at public health centers tends to increase during the Covid-19 pandemic. Medical waste management practices not in accordance with the procedures can be a source of infection. It is necessary to increase the skills of health center staff in managing medical THM waste. The purpose of this study was to determine the level of knowledge and skills of health center staff in medical THM waste management before and after medical THM waste management training. The training participants were 20 health center staff who filled out a pretest questionnaire to measure their level of knowledge and skills prior to the training. The training materials included THM waste, medical THM waste, medical THM waste management and medical THM Temporary Storage. After the training, a post-test was conducted to measure the knowledge and skills scores of the trainees. The results of this study indicated that there was a significant difference in knowledge before and after training ($p < 0.05$). The average knowledge score before training was 6.2 (sufficient knowledge) and after training 8.15 (good knowledge). There was a significant difference in skill scores before and after training ($p < 0.05$). The average skill before training was 6.3 (adequate) and after training 8.65 (good). In conclusion, there was an increase in the skills of health center staff in managing medical THM waste after participating in medical THM waste management training.

Keywords: *Public health center; public health; medical THM waste management; Covid-19 pandemic; training; skill upgrade*

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Highlights:

1. There was an increase in the accumulation of B3 waste in health centers during the COVID-19 pandemic.
2. The knowledge and skills level of health center staff in medical THM waste management before and after medical THM waste management training were determined.
3. Medical THM waste management training was increasing the knowledge and skills level of health center staff.

INTRODUCTION

Puskesmas or public health center is the spearhead of health services in Indonesia, which provides public health services as well as health services for individuals. The role of the health center is very important in preventing and controlling Covid-19 in Indonesia (Rahayu & Sahli 2020). The health center organizes Individual Health Efforts (IHE) related to Covid-19, including 3T, testing, tracing and treatment (Hakim et al. 2021). As the first level health facility (FLHF), the health center provides first contact services both for Covid-19 and non-Covid-19 patients. It must be able to sort out Covid-19 and non-Covid-19 patients by conducting testing and taking swab sampling (Lindner et al. 2021). Likewise, if a positive Covid-19 patient is found, the health center should follow up with tracing (Wan et al. 2021), conducting contact surveys to families or communities exposed to Covid-19 (Sitompul et al. 2021). As FLHF, the health center also carries out a treatment, provides therapy to Covid-19 patients with mild to moderate symptoms, and makes referrals to Advanced Health Facilities (ALHF) according to the condition of Covid-19 patients.

In carrying out Community Health Efforts (CHE), the health center plays a very important role in the efforts to break the chain of transmission of Covid-19 by empowering the community to do 3M (wear masks, wash hands with soap and keep a distance by implementing Clean and Healthy Lifestyle (CHLB)) from, by and for the community by involving partnerships and across sectors (Fibriana et al. 2021). In carrying out all Covid-19 prevention and control activities at health centers, it is necessary to pay attention to the safety aspects of the staff by providing Personal Protective Equipment (PPE) (Chand et al. 2021, McCarthy et al. 2020, Pangihutan 2019), in accordance with the standards, including headgear, masks, gloves and protective gowns. Some of these PPEs are disposable, so that the generation of medical THM waste at the health center tends to increase in the Covid-19 pandemic era (Prihartanto 2020, Subhi 2020).

According to the Ministry of Health, only 6.89% of the health centers have medical waste management practices that meet the standards. There are still many health centers that do not manage waste according to standards (Ministry of Health 2020). Lack of attention to medical waste management practices and practices that are not in accordance with procedures can be a source of the spread of infection (Cut 2015), whereas in one hospital in Bandung only 56% of the health center staff have good knowledge and attitude towards solid medical waste management (Maharani et al. 2017).

Therefore, efforts to improve the skills of health center staff in managing medical THM waste in the Covid-19 pandemic era need to be carried out. The purpose of this study was to identify the level of knowledge and skills of health center staff in managing medical THM waste before and after training on medical THM waste management.

MATERIALS AND METHODS

Public Health Center Songgon is a part of the Technical Implementation Unit, Health Office, Banyuwangi Regency, East Java, Indonesia, which is responsible for health development in its working area. Public Health Center Songgon is a rural health center, a small inpatient health center established in1970. The average number of patients was 85 people per-day for outpatients and 8 people per-day for inpatients. Songgon Health Center already has UKL-UPL documents as guidelines in managing medical THM waste and has collaborated with third parties as Transporters and Processors of medical THM waste. Songgon Health Center produced 2-3 kg of medical THM waste/day. Currently, Songgon Health Center had attempted to obtain a permit for a Temporary Storage Place (TSP) for THM waste to the Environmental Service, Banyuwangi Regency, Indonesia.

Training to improve the skills of health center staff in managing medical THM waste was carried out at Songgon Health Center with 20 participants from the health center staff. The training was carried out by implementing health protocols. The trainees filled out a pretest questionnaire to measure the level of knowledge and skills prior to the training. The training materials presented by the resource persons included THM waste, medical THM waste, medical THM waste management and medical THM Temporary Storage (TPS).

After the training, post-test was conducted to measure the level of knowledge and skills of the trainees. The instrument to measure the level of knowledge consisted of 10 favorable and unfavorable questions with correct and incorrect answer choices, while the skill level was measured using 10 favorable and unfavorable questions with yes and no answers. Knowledge or skill was considered good if the score was more than 7.5, sufficient if the score was 6 and to less than 7.5, and poor if the score was less than 6.

RESULTS

Table 1. Characteristics of Training Participants

Characteristics	n	%
Age		
Mean ± SD	38.3 ± 10.362	
Sex		
Male	8	40
Female	12	60

The average level of knowledge before training was 6.2 (adequate) with the lowest score of 3 and the highest score of 8. The average level of knowledge after training was 8.15 (good) with the lowest score of 4 (less) and the highest score of 10 (good)

Table 2. Distribution of participants with correct answers regarding knowledge

Knowledge	Pre-Test		Post-Test	
	n	%	n	%
Difference between THM waste and medical THM waste	4	20	6	30
Those classified as medical THM waste	12	60	18	90
Color of medical THM waste bag	17	85	16	80
What needs to be put into the safety box	13	65	18	90
Storage time for medical THM waste	15	75	19	95
Sorting of medical THM waste	8	40	15	75
Medical THM waste management	12	60	18	90
Transportation of medical THM waste	18	90	17	85
THM waste temporary storage place requirements	16	80	20	100
Who gives permit for the temporary storage place for THM waste	9	45	16	80

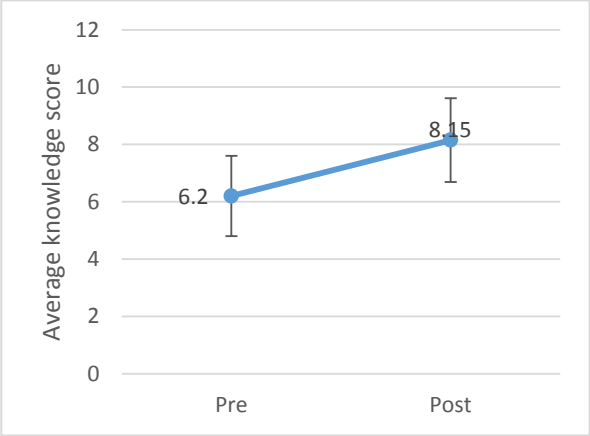

Figure 1. Average knowledge score before and after training

The average skill score before training was 6.3 (adequate) with the lowest score of 2 (less) and the highest of 8 (good). The average skill score after training was 8.65 (good) with the lowest score 8 (good) and the highest 10 (good).

Table 3. Distribution of participants with correct answers regarding skills

Skill	Pre-Test		Post-Test	
	n	%	n	%
Sorting medical THM waste from domestic waste	18	90	20	100
Separate sharp and non-sharp medical THM waste	13	65	19	95
Tying up medical THM waste bags	3	15	8	40
Weighing medical THM waste	13	65	20	100
Wearing PPE when transporting medical THM waste	15	75	19	95
Having medical THM waste management SOP	18	90	20	100
Storing medical THM waste at the THM waste temporary storage	4	20	7	35
Having collaboration with 3rd parties	19	95	20	100
Washing hands with soap when handling medical THM waste	20	100	20	100
Participating in medical THM waste management training	3	15	20	100

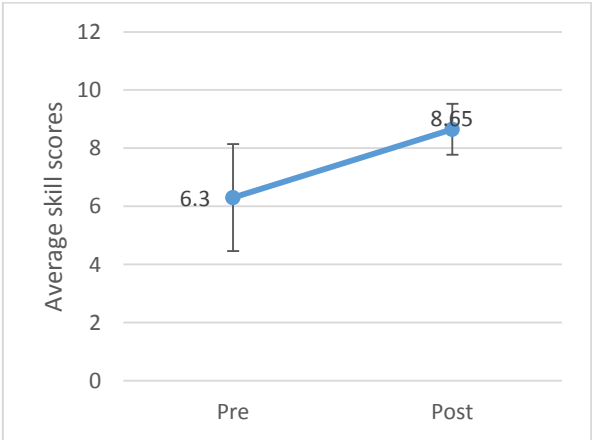

Figure 2. Average skill scores before and after training

Table 4. Testing the normality of the distribution of differences in knowledge and skills data before and after the test

Difference data	n	p-value
Knowledge	20	0.069
Skill	20	0.005

The Shapiro-Wilk test showed that the difference in knowledge scores had a normal distribution ($p > 0.05$). Then to analyze the difference in knowledge before and after training, parametric paired t-test was used, while the data on the difference in skill scores did not have normal distribution ($p < 0.05$). Furthermore, to analyze the differences in skills before and after training, the non-parametric Wilcoxon test was performed.

Table 5. Differences in knowledge before and after training

Knowledge	n	Mean ± SD	Mean ± SD of the Difference	p-value
Pre	20	6.20 ± 1.399	1.95 ± 1.317	< 0.001
Post	20	8.15 ± 1.461		

Paired t-test showed a significant difference in knowledge pre- and post-training ($p < 0.05$).

Table 6. Differences in skill scores before and after training

Skill	n	Median (min – max)	Median (min – max) of the Difference	p-value
Pre	20	7 (2 – 8)	2 (0 – 6)	< 0.001
Post	20	8 (8 – 10)		

The Wilcoxon test showed a significant difference in skill scores before and after training ($p < 0.05$).

DISCUSSION

Before training

Regarding the level of knowledge of Songgon health center staff, less than 50% of the staff already knew the difference between THM waste and medical THM waste (20%), sorting medical THM waste (40%), and the offices that permitted temporary storage of THM waste (45%). Those three topics were related to the topics given in the training, e.g., THM and medical THM waste, medical THM waste management, and temporary storage places for THM waste.

More than 50% of the staff of Songgon health center already knew the classification of medical THM waste (60%), medical THM waste treatment (60%), materials that should be put into the safety box (65%), medical THM waste storage time (75%), requirements of THM waste temporary storage (80%), color of medical THM waste bags (85%) and transportation of medical THM waste (90%). The average level of knowledge of Songgon health center staff regarding the management of medical THM waste before the training was adequate (6.20 ± 1.399).

Regarding skill level, only 15% of Songgon public health centers had attended medical THM waste management training. In the management of medical THM waste, the skills that were still lacking were in the binding of medical THM waste bags (15%) and storage of medical THM waste in THM waste temporary storage (20%). Staff of Songgon health center were skilled in separating sharp and non-sharp medical THM waste (65%), measuring the weight of medical THM waste (65%), wearing PPE when transporting medical THM waste (75%), sorting medical THM waste from domestic waste (90%), had SOPs for medical THM waste management (90%), had cooperation with third parties (95%) and washing hands with soap when handling medical THM waste (100%). The average skill score of Songgon health center staff before the training was 6.3 (adequate).

Training materials

Waste is the residue of an undertaking activity, and can cause severe hazardous impact on human life (Ayilara et al. 2020). Toxic and Hazardous Materials (THM) are substances, energy, and/or other components which, due to their nature and/or concentration and/or amount, either directly or indirectly, can pollute and or damage the environment (Barinova et al. 2019), and can harm the environment, life, health, survival of humans and other living beings (Ferronato & Torretta 2019). Toxic and Hazardous Materials (THM) waste is waste containing THM (Article 1 Regulation of the Minister of Environment and Forestry 56/2015).

Medical THM waste is residual goods or materials resulted from activities that are not reused which have the potential to be contaminated by infectious substances or in contact with patients and/ or staff at health care facilities who treat patients, including used masks, used gloves, used bandages, used tissue, used food and beverage plastic container, used food and beverage paper, used syringes, used infusion sets, used PPE, patients' food scraps and others, originating from service activities in the ER, isolation rooms, ICU rooms, treatment rooms, and other service rooms (Ministry of Health 2020).

Waste originating from pharmaceuticals that have no contact with patients and staff that has the potential to be contaminated with infectious substances, is included in THM waste, instead of medical THM waste. THM waste management from health care facilities "… *is a series of activities that include reduction and sorting, storage, transportation, burial, and/or landfill*" (Article 5 of the Regulation of the Minister of Environment and Forestry 56/2015).

The reduction and sorting of THM waste was carried out by avoiding the use of THM waste materials; good management of materials that had the potential to cause health/ environmental disturbances; good management in the procurement of chemicals and pharmaceuticals to avoid accumulation and expiration; periodic maintenance of equipment; sorting according to type, group, characteristics of THM waste; and packaging according to the THM waste group. THM waste sorting was carried out as early as possible, starting from planning, activating up to THM waste temporary storage, not only when THM waste was stored temporarily.

THM waste storage was carried out by the producer in a temporary storage area for THM waste; the color of the packaging/ container corresponded to the waste (red for radioactive waste, yellow for infectious and pathological waste, purple for cytotoxic waste, brown for expired chemicals, spills or packaging residue). Medical THM waste includes infectious and pathological waste, so that the color of the packaging/container/plastic was yellow. In addition to medical THM waste, the health center was also the producer of domestic waste packaged in black plastic containers (Puangmanee & Jearanai 2020).

THM waste storage time depends on the type of THM waste; medical THM waste for 2 days (storage at temperature $> 0^0$ C), 90 days (storage at temperature $\leq 0^0$ C); expired chemicals, spills, packaging residues, radioactivity, pharmaceuticals, cytotoxics, medical equipment with high heavy metals, and gas cylinders for 90 days (for THM waste generated ≥50 kg/day), and 180 days (for THM waste generated ≤50 kg/day).

If the producer does not have THM waste temporary storage, the storage time is only 2 days. For this reason, the health center needs a freezer, so that they can store medical THM waste at a temperature of $\leq 0^0$ C. Besides, the storage time for medical THM waste can be a maximum of 90 days or 3 months. This is related to collaboration with third parties in terms of transportation and processing of medical THM waste, which will have an impact on financing.

THM waste transportation is carried out by producers and transporters who have transportation permits. If the health center as a THM waste producer does not have a THM waste transport permit, then the health center cannot act as a THM waste carrier. The health center can only transfer THM waste from ER, inpatient, outpatient, and others to THM waste temporary storage place.

The management of medical THM waste is as follows: medical THM waste is put into a container/ bin lined with a yellow plastic bag. After full, THM waste is packed and tied tightly; must be transported daily, recorded and stored at THM waste temporary storage; collection of medical THM waste to THM waste temporary storage is carried out using special infectious waste transportation means and officers who are using PPE.

During Covid-19 pandemic, in the temporary storage place for THM waste, the packaging of THM Covid-19 waste is disinfected by spraying disinfectant on the waste bags that have been wrapped. After use, the container/bin is disinfected with disinfectants such as 0.5% chlorine, lysol, carbolic acid, and others. The transport officer who has finished work takes off his PPE and immediately takes a shower using antiseptic soap and running water. Disinfection with 0.5% chlorine disinfectant in THM waste temporary storage place is carried out thoroughly, at least once a day.

Processing can use the services of a licensed processing company, by entering into a cooperation agreement with a third party. THM waste generation/volume must be recorded in a logbook every day; THM waste manifest must be owned; the amount of medical THM waste must be reported to the Ministry of Environment and Forestry through the Provincial/Regency/City Environmental Service electronically.

Temporary storage place (TPS) for THM waste is a place used to store THM waste if processing cannot be carried out, to prevent the release of THM waste into the environment, so that potential hazards to the environment can be avoided. Everyone who generates THM waste is required to manage the THM waste they produce (Article 3 of Government Regulation no. 101 of 2014).

Temporary storage of THM waste must meet the requirements. The location must be free from flooding and not prone to natural disasters and must be under the control of THM waste generators, as evidenced by a Building Permit (IMB). Waste storage facilities are in accordance with the amount of THM waste and the characteristics of THM waste and are equipped with efforts to control environmental pollution as well as the presence of emergency equipment and management

which includes a Light Fire Extinguisher (APAR), emergency response equipment, first aid kit, and sink.

THM waste storage facilities can be in the form of permanent buildings with designs and constructions, capable of protecting from rain and heat, lighting and ventilation and doors, as well as location instructions affixed to doors; the electrical outlet is outside the building; the container is adjusted to the amount and type of THM waste, and there is a separation between sharp and non-sharp solid THM waste. The permit for temporary storage of medical THM waste is issued by the Regency/ City Environmental Service (DLH).

After Training

There was a significant difference in knowledge before and after training ($p < 0.05$). The average level of knowledge before training was 6.2 (adequate) with the lowest score of 3 (less) and the highest score of 8 (good). The average level of knowledge after training was 8.15 (good) with the lowest score 4 (less) and the highest score 10 (good).

There was a significant difference in skill scores before and after training ($p < 0.05$). The average skill score before training was 6.3 (adequate) with the lowest score of 2 (less) and the highest of 8 (good). The average skill score after training was 8.65 (good) with the lowest score 8 (good) and the highest 10 (good). The training has provided education about behavioral changes at psychomotor level, so that the increase in skill scores was better than the increase in knowledge scores. Before the training, there were participants with low skill scores, while after the training all participants had good skill scores.

Strength and limitation

The study demonstrates the effectiveness of medical THM waste management training in improving the knowledge and skills of health center staff in managing medical waste during the Covid-19 pandemic. The study's findings can inform the development of more comprehensive training programs and policies to promote proper management of medical waste in health centers to minimize the risk of infection transmission. Furthermore, the study highlights the need for health centers to have adequate medical waste management practices that meet the standards to prevent the spread of infections.

CONCLUSION

There was an increase in the skill of health center staff in managing medical THM waste in Covid-19 pandemic era after receiving training on medical THM waste management by the Community Service Team of the Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. Medical THM waste management is a shared responsibility of health care facilities, academics and local governments.

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Conflict of interest

None.

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Author contribution

LDj, LD, S, AIM, and ACF contributed to design's study, analysis data, and prepared the of the manuscript. LD was write the manuscript. LDj was check the grammar, final revised, and final agreement content.

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Original Research

EFFECT OF LONG-TERM KETOGENIC DIET IN MICE SERUM ADIPONECTIN

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ABSTRACT

Ketogenic diet is a popular diet to reduce weight gain quickly. This diet has become a lifestyle. The ketogenic diet has been reported to affect adiponectin, although it is still contraindicated. Adiponectin is a biomarker for a metabolic disease that plays an important role as a protective factor for cardiovascular disease and increases insulin sensitivity. This study aimed to determine the long-term effect of ketogenic diet on adiponectin in mice. This study was an experimental laboratory study with a randomized posttest-only control group design. Fourteen male mice aged 2-3 months (20-30 g) were divided randomly into SD (n=7, standard diet) and KD (n=7, ketogenic diet), given a diet for eight weeks and ad libitum. Bodyweight was measured pre- and post-intervention, whereas adiponectin was measured post-intervention using ELISA. Significant difference of weight gain (Δ) on SD (12.00±6.26) g, KD (1.29±7.41) g with p<0.005. There was a significant difference of serum adiponectin on SD (0.082±0.014) µg/ml and KD (0.096±0.008) µg/ml with p<0.005. This study showed ketogenic diet-induced higher serum adiponectin and slower weight gain. There was no correlation between the difference in body weight and serum adiponectin (p>0.005).

Keywords: *Adiponectin level; ketogenic diet; weight gain; obesity*

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Highlights:

1. Adiponectin is an important protective factor for cardiovascular disease and increased insulin sensitivity.
2. Ketogenic diet effect on adiponectin level in mice besides decrease weight gain was determined.
3. The difference in serum adiponectin level and body weight is uncorrelated.

INTRODUCTION

The ketogenic diet is a diet strategy with a composition of high fat, low carbohydrates, and sufficient protein, which is more popular than other diets (Li et al. 2020, Walczyk & Wick 2017). This diet can overcome overweight and obesity quickly (Castellana et al. 2020). Li et al. (2020) stated that the ketogenic diet effectively treats epileptic seizures, metabolic disorders, tumors, autosomal dominant polycystic kidney disease, and neurodegeneration. In addition to its usefulness for non-pharmacological therapy, this diet has become popular because it is healthier than currently recommended (Kirkpatrick et al. 2019). The public increasingly recognizes *ketofastosis* lifestyle in Indonesia with number of users in 2016. *Ketofastosis* uses ketogenic diet and fasting to maintain health (Fatimah & Husniawati 2019).

The use of ketogenic diet has been reported to affect adiponectin. Adiponectin is a hormone produced by adipose tissue and is a biomarker of metabolic disease (Fang & Judd 2018, Li et al. 2020). Adiponectin decreased significantly in obese patients (negatively correlated with BMI), type 2 diabetes mellitus (DMT2) patients (regardless of BMI), and coronary artery disease (CAD) patients. Adiponectin is a significant cardiovascular disease protective factor, because it improves insulin sensitivity, improves postprandial glucose and lipid metabolism, and also has anti-inflammatory, anti-atherogenic, and anti-angiogenic properties (Balsan et al. 2015, Monda et al. 2020).

The effect of the ketogenic diet on adiponectin is known to be contraindicated (Asrih et al. 2015, Monda et al. 2020, Partsalaki et al. 2012, Sena et al. 2017). Even though the use of ketogenic diet is already widespread in Indonesia (Fatimah & Husniawati 2019), because it can prevent overweight and obesity. The preventive effects resulting from ketogenic diet through adiponectin, such as protection against cardiovascular disease and increasing insulin sensitivity, may also contribute to the use of this diet. Therefore, a study explored the long-term effect of a ketogenic diet to increase serum adiponectin in mice.

MATERIALS AND METHODS

It was an experimental laboratory study with a randomized posttest-only control group design using fourteen male mice, DDY strains, aged 2-3 months, 20-30 g, as subjects. The subjects were acclimatized for one week, given a standard diet ad libitum. For the next eight weeks, the control group (SD) was given a standard diet (n=7) and the ketogenic group (KD) was given a ketogenic diet (n=7), ad libitum.

The composition of the standard diet was 20% protein, 12% fat, and 62% carbohydrate. The composition of the ketogenic diet was 30% protein, 60% fat, and 0% carbohydrate.

This study was approved by the Research Ethics Committee, Faculty of Medicine, University Airlangga No. 256/EC/KEPK/FKUA/2020. The study took nine weeks at the Biochemistry Animal Laboratory, Faculty of Medicine, Universitas Airlangga.

Bodyweight measurement

Bodyweight was measured at pre- and post-intervention using Harnic HL-3650 Heles Digital Scale which maximizes 5 kg and division graduation of 1 g.

Adiponectin measurement

The cardiac puncture procedure was used to collect blood samples 24 hours after the last meal. Blood samples were centrifuged at 4000 rpm for 5 minutes to obtain serum samples. Serum adiponectin were measured post intervention using an Enzyme-Linked Immunosorbent Assay (ELISA) kit, catalog No E-EL-M0002. The specification was sensitivity up to 9.38 pg/mL and detection range 15.63-1000 pg/mL.

Statistical analysis

Data analysis was performed using Statistical Package for Social Sciences (SPSS) program version 16. Data were presented in numbers and percentages. Numerical data were presented in the mean (standard deviation) and (standard error) if the data were normal. The Shapiro Wilk test was used to determine normality. Independent t-test was used to determine mean difference for normal distribution and Mann Whitney test was used for abnormal distribution. Pearson Correlation was used to determine correlation between bodyweight and serum adiponectin level. The statistical significance was $p<0.05$.

RESULTS

Fourteen male mice, DDY strains, aged 2-3 months, 20-30 grams were divided into control group (SD) and ketogenic group (KD). Pre- and post-intervention bodyweight was normally distributed ($p>0.05$). Since there is a connection between pre- and post-intervention bodyweight, a difference of bodyweight analysis was performed. The differences of body weight were not normally distributed ($p<0.05$). Furthermore, Mann-Whitney comparative test was performed. The characteristics of the body weight of subjects reported at Table 1. Adiponectin of KD (0.096±0.008 µg/ml) was reported significantly different than SD (0.082±0.014 µg/ml) with p-value 0.035 (Figure 1). The difference of bodyweight and adiponectin were tested using Pearson's correlation. The p-value of 0.403 was assumed that difference of bodyweight and serum adiponectin in the standard and ketogenic diet had no relationship.

Table 1. Characteristics body weight of subjects

Bodyweight (g)	SD (7)	KD (7)	Comparative test
Pre-intervention	24.29±3.64	26.71±2.87	0.19
Post-intervention	36.29±6.75	28.00±9.49	0.08
Difference (Δ)	12.00±6.26	1.29±7.41	0.02*

Abbreviation: SD=Control Group; KD=Ketogenic Group

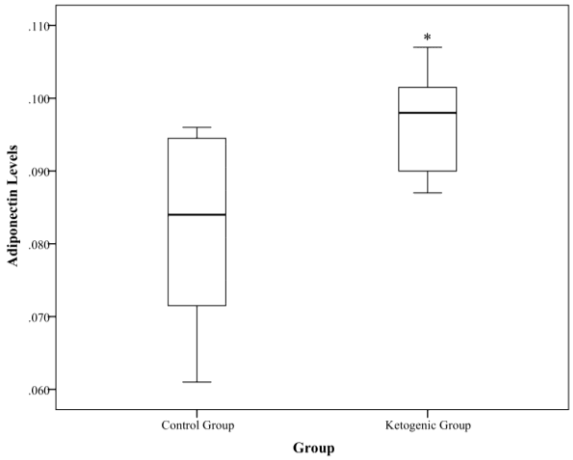

Figure 1. Serum adiponectin levels significantly increased in ketogenic group (p=0.035)

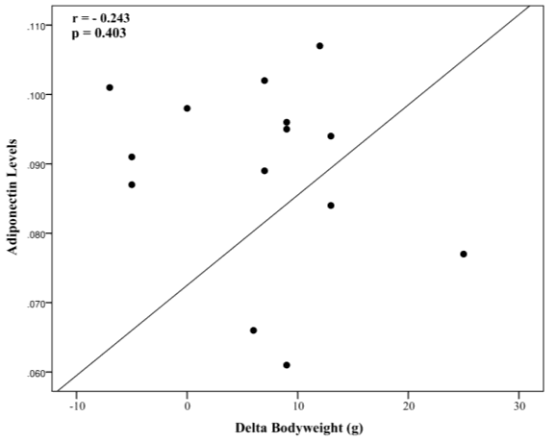

Figure 2. Serum adiponectin levels were not correlated with difference of bodyweight

DISCUSSION

Ketogenic diet consists of a high fat, low carbohydrate, and sufficient protein (Li et al. 2020). In benzopyrene-induced mice, ketogenic diet with a fat has protein ratio of 60 : 30 that can reduce weight gain (Utami et al. 2021). The high-fat diet group with a composition of 30% protein, 60% fat, and 0% carbohydrate experienced the most weight loss and had the least amount of visceral fat compared to other diet groups (Syahraya et al. 2020). In condition with lack of carbohydrates, body will carry out gluconeogenesis to provide adequate energy. When endogenous glucose production does not meet body's needs, ketogenesis begins to take over to provide an alternative energy supply (Sherrier & Li 2019). The absence of compensation in low glucose conditions causes a decrease in insulin and an increase in glucagon, which leads to increased ketogenesis (Widiatmaja et al. 2021). Ketogenesis results in ketone bodies: acetyl CoA, β-hydroxybutyrate (βHB), and acetone, which lead to ketosis (Paoli 2014).

Ketone bodies production activates *hypothalamic ventromedial nucleus*, which is directly related to satiety, suppressing appetite, and leads to weight loss (Monda et al. 2020). A previous study revealed that people with ketogenic diet felt significantly less hungry (p=0.014) (p=0.014) (Gershuni et al. 2018). Ketogenic diet participants tend to maintain lean body mass with a decrease in preferential fat mass, regardless of exercise (Gershuni et al. 2018). There was no statistically significant difference in daily energy expenditure between ketogenic and standard diets (Hall 2019). After 12 weeks of ketogenic diet, there was a decrease in bone volume fraction, cancellous bone trabecular number, cortical thickness, total cross-sectional area in the periosteal envelope, and cortical bone area in the tibia and humerus, while trabecular separation increased (Ding et al. 2019).

Monda et al. (2020) used twenty obese subjects consisting of 10 women and men aged 20 to 60 years who were fed a very low-carbohydrate ketogenic diet (VLCKD) for eight weeks. Adiponectin was found to be 10.8±1.2 g/ml at the beginning of the study. Adiponectin became 25.55±1.3 µg/ml with p value<0.001 after the intervention of VLCKD (Monda et al. 2020). Adiponectin increased significantly after VLCKD intervention. Another study demonstrated a significant difference in adiponectin between the control and high-fat diet groups (HFD). Sena et al. (2017) fed 12-month-old male Wistar rats HFD (40% triglycerides and 10% carbohydrates) and a standard diet (5% triglycerides and 45% carbohydrates) for four months. At the end of the study, adiponectin in the standard diet group was 43.99±6.0 g/ml and 46.15±4.37 g/ml in the HFD group with p<0.05. This previous study demonstrates that exposing rodents to ketogenic diet for four months can increase adiponectin level compared to standard diet. The results of this study were in accordance with previous research, where there was significant difference in serum adiponectin between KD and SD.

Decreased ROS and improved mitochondrial function are the effects of carbohydrate restriction which induces stress response proteins. By reducing coenzyme Q, ketone bodies induce a decrease in free radical production (Veyrat-Durebex et al. 2018).The increased mitochondrial biogenesis function also increases adiponectin synthesis (Fang & Judd 2018).

The ketogenic diet produces βHB which induces adiponectin secretion through G Protein-Coupled Receptor 109A (GPR109A) (Li et al. 2020, Plaisance et al. 2009). Fatty acids activate peroxisome Proliferator-Activated Receptor Alpha (PPARα), and it inhibits pro-inflammatory cytokines including IL-6 and Tumor Necrosis Factor Alpha (TNFα) (Veyrat-Durebex et al. 2018). TNFα and IL-6 can inhibit adiponectin gene expression and adiponectin secretion from 3T3-L1 adiposity (Fang & Judd 2018). The inflammatory cytokine TNFα also interferes with Fibroblast Growth Factor 21 (FGF21) through β-Clotho regulation. A ketogenic diet induces hepatic insulin resistance and increases the level of FGF21, increasing adiponectin secretion production by targeting adipose tissue and mediating systemic effects (Asrih et al. 2015).

Sena et al. (2017) explain that adiponectin has pleiotropic action which improves endothelial dysfunction through reducing production ROS, promoting coupling and activity of Endothelial Nitric Oxide Synthase (eNOS), increasing NO availability, and inhibiting JNK pro-inflammatory kinase. Adiponectin has anti-inflammatory, anti-atherogenic, and anti-angiogenic functions (Monda et al. 2020). Adiponectin stimulates oxidation of fatty acid in muscle by increasing the expression of molecules involved in fatty acid transport (CD36), their combustion (acetyl Co-A oxidase), and energy dissipation through an increased expression of type-2 release protein (Uncoupling Protein 2/UCP-2) (von Frankenberg et al. 2017).

Strength and limitation

The study uses a randomized controlled design, which is a strong study design that minimizes bias and allows for comparison of the intervention and control groups.

CONCLUSION

A long-term ketogenic diet, consisting of 30% protein, 60% fat, and 0% carbohydrate, induced higher serum adiponectin, and slowed down weight gain, although there was no correlation between weight gain and serum adiponectin levels.

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Conflict of interest

None.

Funding disclosure

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Author contribution

HK, HKh, and SS were conseptual, prepared the data analysis of the manuscript, revised. PSR final check the manuscript and grammar.

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Original Research

ACUTE DIARRHEA PATIENTS AMONG CHILDREN UNDER FIVE HOSPITALIZED IN A TERTIARY HOSPITAL IN EAST JAVA, INDONESIA

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ABSTRACT

Diarrhea is the second leading cause of morbidity and mortality in children under five years of age in Indonesia after pneumonia. In 2017, diarrhea became an outbreak in 12 provinces. Diarrhea in under-fives can cause several complications and can cause death if it is not treated properly. This study aimed to evaluate the characteristics of acute diarrhea patients in under-fives hospitalized in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia for six months. This study was a descriptive study with retrospective approach which evaluated all acute diarrhea patients hospitalized from July to December 2019. Patients' data were taken from medical records and presented descriptively. Of the total 125 patients, most acute diarrhea patients were male (60%) aged 0 to 24 months (83.2%), had good nutritional status (58.4%), and hospitalized for less than 5 days (60.8%). The most degree of dehydration was mild-moderate dehydration (83.2%), mostly treated with intravenous rehydration (96.8%), the most common comorbidity was anemia (13.4%), and the most electrolyte disorder was hyponatremia (41%), while acid-base disorder was mostly metabolic acidosis (75%).

Keywords: *Diarrhea; acute diarrhea management; tropical disease; child health; pediatric*

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Highlights:

1. The characteristics of acute diarrhea patients among chidren in Tertiary Hospital In East Java was evaluated.
2. The highest incidence of diarrhea was found in the 0-24 months age group, good nutritional status, male sex, and hospitalized in less than 5 days.
3. Mild-moderate dehydration, intravenous rehydration treated, anemia, hyponatremia, and metabolic acidosis were mostly founded.

INTRODUCTION

Diarrhea is a liquid or unformed stools associated with increased frequency of defecation. The increased frequency is defined by three or more bowel movements a day (Rao et al. 2014). The data of the Ministry of Health, Indonesia, in 2020 indicated that diarrhea was the second cause of morbidity and mortality of under-fives in Indonesia after pneumonia. The number of infant and under-fives deaths caused by diarrhea was 760.000 every year, and dominantly occurred in children under five. In addition, 21% of deaths of children in developing countries was caused of diarrhea (World Health Organization 2013). In 2017, diarrhea had become an outbreak in 12 provinces (Ministry of Health 2018).

Diarrhea is still a public health problem in developing countries, such as Indonesia. It was due to its high morbidity and mortality. Therefore, it is necessary to identify the appropriate characteristics of acute diarrhea in order to provide an optimal treatment or therapy results before causing complications. We evaluated the characteristics of acute diarrhea in children under five years old hospitalized at Dr. Soetomo General Academic Hospital, Surabaya.

MATERIALS AND METHODS

A retrospective study was conducted at the Department of Pediatrics in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, from July to December 2019. The subjects were children diagnosed with acute diarrhea. The inclusion criteria were children aged 0-60 months with main acute diarrhea diagnosis in the hospital, while the exclusion criteria were children aged 0-60 months with the main acute diarrhea diagnosis and undergoing hospitalization with incomplete and illegible medical records. The instrument of the study was medical record of patients.

There were 125 subjects obtained through total sampling method. The data were classified as participant's age, gender, nutritional status, length of stay, given therapy, diarrhea with complications of dehydration (degree of dehydration), electrolyte imbalance, acid-base imbalance, and with comorbidities.

Data retrieval on nutritional status variables was determined based on z-score criteria. Data collection on degree of dehydration was categorized based on WHO criteria. All data were collected and analyzed using IBM SPSS. This research had been approved by the ethics committee of Dr. Soetomo General Academic Hospital with Decree No. 0211/LOE/301.4.2/XI/2020.

RESULTS

There were 143 patients with the main acute diarrhea diagnosis treated at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia from July to December 2019. After considering the inclusion and exclusion criteria, 125 patient data met the criteria and could be used as research samples. A total of 18 patients were excluded due to incomplete and illegible medical records.

Table 1. Distribution of sociodemographic characteristics in patients with acute diarrhea

Sociodemographic Characteristics	Frequency (n)	Percentage (%)
Age		
0-24 months	104	83.2
25-36 months	6	4.8
37-60 months	15	12
Gender		
Male	75	60
Female	50	40
Nutritional status		
Good nutrition	73	58.4
Lack nutrition	22	17.6
Malnutrition	26	20.8
More nutrition	4	3.2
Length of stay		
Less than five days	76	60.8
Equal to or more than five days	49	39.2

In this study, diarrhea patients in children under-five were highest at 0-24 months of age (83.2%), had male sex (60%), mostly had good nutritional status (58.4%), and were hospitalized for less than five days (60.8%) (Table 1).

Table 2. Distribution of clinical characteristics in patients with acute diarrhea

Clinical Characteristics	Frequency (n)	Percentage (%)
Degree of dehydration		
Without dehydration	4	3.2
Mild-moderate dehydration	104	83.2
Severe dehydration	17	13.6
Therapy		
Intravenous rehydration		
Yes	121	96.8
No	4	3.2
Antibiotics		
Yes	40	32
No	85	68
Zinc		
Yes	111	88.8
No	14	11.2
Probiotics		
Yes	86	68.8
No	39	31.2
Comorbidities		
Pneumonia	7	5.5
Anemia	17	13.4
Febrile seizure	2	1.6
Without comorbidities	101	79.5

In this study, almost all patients (96.8%) had dehydration complications, while 4 patients (3.2%) were not dehydrated. A total of 104 patients (83.2%) had acute diarrhea with mild to moderate dehydration, while 17

patients (13.6%) had acute diarrhea with severe dehydration. As much as 96.8% of the patients were given *intravenous rehydration* therapy. Meanwhile, 40 (32%) patients were given antibiotic therapy, 111 (88.8%) patients were given zinc, and 86 (68.6%) patients were given probiotics. Acute diarrhea patients who had pneumonia as many as 7 patients (5.5%), anemia as many as 17 patients (13.4%), and febrile seizures as many as 2 patients (1.6%), while those who did not have comorbidities were as many as 101 patients (79.5%). One patient with acute diarrhea could have more than one comorbidity.

Table 3. Laboratory characteristics distribution in patients with acute diarrhea

Laboratory Characteristics	Frequency (n)	Percentage (%)
Electrolyte disturbance		
Yes	37	29.6
Hyponatremia	18	41
Hypernatremia	5	11.4
Hypokalemia	17	38.6
Hyperkalemia	4	9
No	25	20.0
Untested	63	50.4
Acid-base disturbance		
Yes	12	9.6
Metabolic acidosis	9	75
Respiratory acidosis	3	25
No	7	5.6
Untested	106	84.8

A total of 29.6% patients had electrolyte disturbances complications and 9.6% of patients had acid-base disturbances complications. In patients with electrolyte disturbances complications, 41% of them had acute diarrhea with hyponatremia complications, 11.4% had hypernatremia complications, 38.6% had hypokalemia complications, while 9% of patients had acute diarrhea with hyperkalemia complications. Twelve patients who had acute diarrhea with acid-base disturbances, 75% of the patients had acute diarrhea with metabolic acidosis complications, while 25% of the patients were accompanied by respiratory acidosis. One acute diarrhea patient could have two balance disorders at the same time.

DISCUSSION

The pathophysiology of diarrhea includes osmotic, secretory, and inflammatory diarrhea. Osmotic diarrhea occurs when absorbable solutes, such as lactose are not properly absorbed and water is retained in the intestinal lumen. Infections that damage intestinal epithelial cells, either directly or through toxins, cause malabsorption and osmotic diarrhea. Secretory diarrhea results from toxin-mediated active secretion of water into the intestinal lumen. It has been observed that during cholera infection, Shiga toxin is produced by *E.coli* and *Shigella* species. Diarrhea can be caused by intestinal inflammation associated with infection. After ingestion, enteric organisms attach to enterocytes and form colonies in the intestinal epithelium. One of two pathways is generally followed depending on the pathogen; either mucosal invasion or enterotoxin production.

The studies showed that the incidence of diarrhea in under-five patients hospitalized at Dr. Soetomo General Academic Hospital, Surabaya, was highest at the age of 0-24 months (83.2%). This study had similar results with a study conducted by Maryanti et al (2017), that the age group under 3 years were affected by diarrhea more. This could be caused by incomplete formation of enzymes in children under 2 years, so that food absorption was less than optimal (Behrman et al. 2000). In general, children less than 2 years of age tend to put their hands or other objects in their mouths, so that poor hygiene levels could increase the risk of diarrhea in children.

Acute diarrhea patients in children under five were dominated by male patients (60%). In a study conducted by Selvia (2017), the incidence of diarrhea mostly occurred in male under-fives (63.3%). The exact cause of this was unknown currently; possibly because boys are more-active than girls, making it easier to be exposed to diarrhea-causing agents (Sujana 2014). The nutritional status of acute diarrhea patients was mostly in a good nutritional status (58.4%). Diarrhea could occur in all groups of children, including children who had good nutritional status. Iskandar (2015) found no significant relationship between nutritional status and diarrhea incidence, because most of the causes of acute diarrhea are viruses transmitted through poor sanitation and hygiene.

In addition, most acute diarrhea patients had a length of stay for less than five days (60.8%). The similar results were obtained in a study that more patients were hospitalized for less than five days (90.4%) compared to patients who were hospitalized more than equal to five days (9.6%) (Yusuf 2011). According to Widiantari and Widarsa (2013), the length of stay for acute diarrhea was determined by the duration of diarrhea, the severity of the disease, and a history of recurrent illness which affected the healing process and the restoration of intestinal mucosal function.

Based on the degree of dehydration of diarrhea patients in children under five, the most was mild to moderate dehydration as much as 83.2%. This was in accordance with a study by Wibisono et al. (2015) that obtained more degrees of dehydration at mild-moderate degrees of

dehydration, as many as 26 children (86.7%). The higher the frequency of diarrhea, the more the excretion of the fluids, which can cause dehydration.

In this study, the most under five acute diarrhea patients used parenteral rehydration fluid therapy. Asyikin (2017) stated that parenteral rehydration was the main therapy in diarrhea patients. The main treatment for diarrhea was to provide a replacement fluid using electrolyte fluids (Ministry of Health 2010). In this study, as much as 32% of acute diarrhea patients received antibiotic therapy. Antibiotics were needed in diarrhea patients to treat infections caused by bacteria and fungi. Inappropriate use of antibiotics could kill the normal flora needed by the children's body, so that it had a higher risk of adverse effects due to bacterial infections caused by incomplete formation of the child's immune system. It would also incur much more unnecessary medical expenses (Asyikin 2017).

Antibiotics therapy in acute diarrhea cases in children did not provide a significant improvement in clinical outcome, because the cause of diarrhea could not always be treated with antibiotics (Trisnowati et al. 2017). Soenarto et al. (2009) stated that rotavirus infection was the main cause of diarrhea in under-fives. In this study, 88.8% of acute diarrhea patients received zinc therapy. These results were similar to a study conducted by Asyikin (2017) that zinc therapy was given to 71.23% of patients with acute diarrhea. Zinc administration as a diarrhea therapy could prevent complications. In the acute diarrhea treatment, zinc could reduce diarrhea episodes duration, and reduce the frequency and volume of stools (Siswidiasari et al. 2014).

In this study, acute diarrhea treatment using probiotics were 86 cases (68.8%). Probiotics are living microorganisms that are intended to have health benefits when consumed or applied to the body by improving balance of intestinal microflora (Siswidiasari et al. 2014). In cases of acute diarrhea in children, probiotics had been widely used, but it had not been recommended by the WHO. In a study, probiotic supplementation was proven to be effective and significant in reducing the duration of diarrhea compared to patients who only received standard rehydration and zinc therapy alone (Mulyani et al. 2016). Probiotics could be used as an effective adjunct therapy in acute diarrhea infections and reduce the frequency and duration of diarrhea so as to minimize the economic burden by decreasing the length of stay (Asyikin 2017).

This study had also indicated that the most common diseases that accompanied diarrhea were anemia (13.4%), followed by pneumonia (5.5%), and febrile seizures (1.6%). Franca et al. (2009) stated that diarrhea accompanied by anemia could be associated with risk factors for diarrhea, such as malnutrition. Malnutrition could interfere with hematopoiesis process which causes anemia due to bone marrow atrophy. Therefore, children who suffered from iron deficiency anemia would be more susceptible to microorganisms (Pratama 2016). Diarrhea accompanied by acute lower respiratory tract infections was the highest cause of morbidity and mortality in children under-five. Co-infection in children was more commonly suspected because incomplete formation of the child's immune system. Several studies have shown that diarrhea and pneumonia often occur together (Walker et al. 2013).

Dehydration became a factor associated with the incidence of seizures in diarrhea patients. Patients with dehydration would have a lack of fluids and electrolytes which could lead to fever. An increase in body temperature could change the balance of neuron cell membranes and diffusion of potassium and sodium ions in a short time with the result of an electrical discharge. With the help of neurotransmitters, electrical discharge could extend to all cells and surrounding cell membranes, causing seizures (Wibowo et al. 2020). Diarrhea patients who had comorbidities should be treated according to the disease and indications while still prioritizing therapy for fluid stabilization if accompanied by dehydration (Sari 2010).

Rotavirus is the leading cause of life-threatening diarrheal diseases among young children. Rotavirus infection of enterocytes causes the invasion of the virus, the formation of viroplasms (VI), and the release of the virus and its toxin NSP4 (nonstructural protein). Intracellular NSP4 (iNSP4) induces an increase in intracellular Ca2 + mainly through er release and PLC-independent mechanism. NSP4 released from the apical side raises intracellular calcium levels through receptor-mediated PLC-dependent mechanisms.

The increase in calcium caused by NSP4 disrupts microvillus cytoskeleton as well as barrier function, increases the flow of paracellular water and electrolytes, and causes diarrhea (Hodges & Gill 2010). Several mechanisms have been proposed that underlie the decreased resorption function of the epithelium, contributing to the pathogenesis of rotavirus-induced diarrhea. These mechanisms include loss of infected enterocytes and NSP4-mediated disruption of sodium-associated solute carriers involved in reabsorption of large amounts of water under physiological conditions. However, the contribution of reduced epithelial absorption to rotavirus-induced diarrhea is unclear due to the effectiveness of oral rehydration therapy to rapidly correct electrolyte and water loss in children with severe diarrhea caused by rotavirus. Some studies suggest that a

sufficient number of enterocytes have intact sodium glucose cotransporters 1 and that rotavirus does not infect all enterocytes. Another explanation is that rotavirus infection increases epithelial cell turnover, which may promote absorption of oral rehydration solution (Crawford et al. 2017).

A total of 29.6% of acute diarrhea patients had electrolyte disturbances. Electrolyte disturbances in the study were mostly hyponatremia (41%), followed by hypokalemia (38.6%), hypernatremia (11.4%), and hyperkalemia (9%). This was in accordance with research conducted by Alfa (2019), that acute diarrhea patients who had sodium electrolyte disorders occur more in hyponatremia (77.9%) compared to hypernatremia (22.1%). In acute diarrhea patients with potassium electrolyte disorders, more hypokalemia (64.3%) was found than hyperkalemia (35.7%).

The incidence of diarrhea in children was mostly caused by an infection that caused damage to the intestinal epithelium and increased intestinal permeability, thus causing diarrhea. The intestinal epithelium functions in the absorption of body fluids. Most absorption of body fluids by the intestines was regulated by sodium and potassium pumps. This could cause electrolyte disturbances in diarrhea patients, especially in sodium and potassium (Nemeth & Pfleghaar 2020). Wololi et al. (2016) stated that hyponatremia in diarrhea was caused by a combination of water and sodium loss, and water retention to compensate for volume loss. Hypokalemia in acute diarrhea could occur due to loss of potassium from the gastrointestinal tract. Diarrhea in children could cause direct loss of potassium because the concentration of potassium in feces could reach 80-90 mEq/L. Hypokalemia could occur due to vomiting resulting in excessive potassium expenditure in children with diarrhea. Vomiting may caused by metabolic alkalosis or the presence of secondary hyperaldosterone because of arising hypovolemia from vomiting or dehydration. This will increase potassium excretion and causes hypokalemia (Kardalas et al. 2018).

In this study, as many as 12 acute diarrhea patients in children had acid-base disturbances. Most of them had metabolic acidosis, which was as many as 9 patients, while 3 other patients had respiratory acidosis. This was in accordance with research by Jurnalis et al. (2008) which said that metabolic acidosis was the most common complication of acute diarrhea. Metabolic acidosis in diarrhea was caused due to the loss of bicarbonate through the stool. In diarrhea children who had anorexia, there could be an increase in organic acid levels in the blood due to the breakdown of body fat and protein to meet calorie needs. There was a decrease in circulation to the kidneys and tissues in diarrhea patients with severe dehydration which caused impaired excretion of organic acids by the kidneys and accumulation of lactic acid due to tissue hypoxia. It also caused metabolic acidosis in diarrhea patients (Sinuhaji 2007).

Strength and limitation

The study addresses an important public health issue in Indonesia, which is the high prevalence of diarrhea in under-fives.The study can help health professionals and policymakers to better understand the disease and design appropriate interventions. The study is limited by its retrospective design, which means that the researchers relied on medical records for data collection. This may lead to incomplete or inaccurate data.

CONCLUSION

In this study, the highest incidence of diarrhea was found in the 0-24 months age group, male sex, good nutritional status, and hospitalized in less than 5 days. The most degree of dehydration was mild-moderate dehydration, mostly treated with intravenous rehydration, and the most comorbidity was anemia, while the most electrolyte disturbance was hyponatremia, and the acid-base disturbance was mostly metabolic acidosis.

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Conflict of interest

None.

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None.

Author contribution

SMS and S - conseptual, study design, analysis data. AFA- write and revised the manuscript. AFt- validation of all manuscript data.

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Original Research

THE PROGNOSTIC ROLE OF NEUTROPHIL TO LYMPHOCYTE RATIO (NLR) IN TESTICULAR GERM CELL TUMOR (GCT)

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ABSTRACT

The global incidence of testicular cancer is 1-2% from all cancers. The attempts to maintain high therapeutic rates while decreasing the treatment-related side effects and toxicity have become the current concern. However, the reports regarding testicular germ cell tumors (GCT) in Indonesia are limited. Thus, we aimed to evaluate the clinical characteristics of testicular GCT patients undergoing bleomycin, etoposide, and cisplatin (BEP) chemotherapy, as well as their chemotherapy response and side effects. We reported the data of patients with Testicular Germ Cell Tumor from January 2015 to December 2019. Several data were retrieved, including patient demographics, tumor characteristics, treatment, and prognosis outcome. A total of 67 patients with testicular germ cell tumors were included in this study. The mean age was 28.9 years old. The chemotherapy regimens used were four cycles of (BEP) in 36 patients (53.7%), followed by three cycles of BEP in 22 patients (32.8%). Patients with seminoma GCT mostly had a complete response (54.1%), whereas most patients with non-seminoma GCT had progressive disease (47.8%). The multiple logistic regression analysis showed that NLR and S staging were independently associated with the patient's response to chemotherapy (OR 2.14, 95% CI 1.22, 3.78, p <0.01,OR 9.43, 95% CI 1.81, 49.14, p < 0.01). The clinical characteristics and response of testicular GCT patients among Indonesian men showed similarity with the current worldwide data. The NLR could be used as a potential biomarker for prognosis.

***Keywords**: Testicular cancer; germ cell tumor; BEP chemotherapy; cancer*

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Highlights:

1. Neutrophil to lymphocyte ratio (NLR) has been reported by several studies for its role as a biomarker in various diseases, however, the role of NLR in testicular GCT is still unclear.
2. The characteristics and responses of testicular GCT patients among Indonesian men show similarity to other reports worldwide.
3. As a parameter, NLR shows promise to be used as a potential biomarker for prognosis in testicular GCT.

INTRODUCTION

Testicular cancer accounts for approximately 1-2% of all types of cancers globally (Purdue et al. 2005). They are considered rare tumors in the population as a whole, and most commonly found in males between 15 and 40 years old (Ghazarian et al. 2017). The incidence of testicular cancer has also increased in the Caucasian populations with a 7.8 and 6.7 in 100,000 men incidence rate in Western and Northern Europe, respectively, compared to Northern Africa which accounted for 0.6 in 100,000 men (Ferlay et al. 2010). The most common type of testicular carcinoma was germ-cell tumors which accounted for 95% of all testicular carcinoma and further classified into seminomas and non-seminomatous GCS (NSGCT). Current treatment in testicular cancer was based on clinical staging from the International Germ Cell Cancer Collaborative Group (IGCCCG) for planning management strategies. The patients were classified for clinical stages based on radiology and tumor marker examination. The treatment options include surveillance, orchiectomy, chemotherapy based on the clinical stage of IGCCCG classification (Gaddam &

Chesnut 2021). The most used protocol regimen for chemotherapy was Bleomycin, Etoposide, and Cisplatin (BEP). BEP is still considered the best treatment in eligible patients. However, the adverse events might limit the beneficial effect of this regimen.

Despite having a great effectivity and high success rate, several side-effects were noted from BEP chemotherapy, including hypercholesterolemia, hypogonadism, and depression (Lavanderos et al. 2019). Attentions have increased lately on the effects of cancer treatment which attempts to maintain high therapeutic rates while decreasing the treatment-related side effects and toxicity. Treatment associated with side effects and toxicity has become an important issue for this population. Most existing articles reporting on GCT have been limited to a particular country or small groups within the country (Kusler & Poynter 2018).

Understanding the unique profile and response to chemotherapy in a population would allow an effective management strategy to further control and increase the survival rate (Farmanfarma et al. 2018). Identifying potential risk factors that may affect the patient's response to treatment is necessary to develop effective diagnostic and therapeutic strategies. The development of cancer progression was influenced by the tumor microenvironment and host inflammatory response. Several studies reported the neutrophil-to-lymphocyte ratio (NLR) for its role in numerous diseases and an increased ratio of NLR is a poor prognostic factor in testicular cancer (Ohno 2019, Mjaess et al. 2021). Its availability in urban and rural centers as well as its low price make it an appealing alternative for a prognostic biomarker in solid tumors (Zhang et al. 2017, Miyamoto et al. 2018, Prabawa et al. 2019, Yin et al. 2019). However, the evaluation of NLR use in GCT is limited. In Indonesia, reports regarding testicular GCT are highly limited. Thus, we aimed to summarize the clinical characteristics of testicular GCT patients undergoing BEP chemotherapy and analyze the prognostic factors affecting their response to chemotherapy.

MATERIALS AND METHODS

We conducted a retrospective analytical cohort study involving patients with testicular GCT treated with chemotherapy in an Indonesian tertiary hospital between January 2015 and December 2019. We include all patients diagnosed with Testicular Germ Cell Tumours (GCT) using clinical and pathological examination who received chemotherapy with or without surgical intervention. All patients who lost to follow-up and incomplete data were excluded from this study.

The medical records of the patient's visits, operations, and follow-up visits were reviewed retrospectively using the patient's unique hospital identification number. We extracted the data from medical records into a standardized data collection form comprised of patient's age, tumor stage from clinical and pathological examination, regimens and the cycle of chemotherapy, response to chemotherapy, and adverse events related to chemotherapy.

The tumor stage was classified using tumor-node-metastasis (TNM) classification according to the 7th edition of the American Joint Committee on Cancer (AJCC) 'cancer staging manual'. We evaluated serum tumor marker (S) staging by measuring lactate dehydrogenase (LDH), human chorionic gonadotropin (HCG), and alpha-fetoprotein (AFP). We classified the prognostic risk of the metastatic disease patients using the International Germ Cell Cancer Consensus Group (IGCCCG). The chemotherapy-related adverse events were assessed with a particular focus on the patient's symptoms, physical examinations, and routine blood counts and grouped using the classification of Common Terminology Criteria for Adverse Events (CTCAE) v5.0. In this study, we evaluated the patient's clinical response to chemotherapy using criteria, including complete response, partial response, stable disease, and progressive disease.

We summarized categorical data using frequency and percentage and displayed continuous data as mean and standard deviation (SD). All data were collected using spreadsheet software Microsoft excel® in 2021 (Microsoft Corporation, Redmond, WA, USA). In this study, we analyzed factors that were associated with patient's responses after receiving 3 or 4 cycles of chemotherapy. Bivariate analyses were performed using independent t-tests, Mann Whitney tests, and chi-square tests to analyze the differences between patients with complete and non-complete responses. We used multiple logistic regression analysis to find variables that were independently associated with patient's response to the chemotherapy. If the p-value of bivariate analysis was less than 0.25, we included the variables into the multiple logistic regression analysis. Observed associations were displayed as Odds Ratio (OR) with 95% Confidence Interval (95% CI). All analyses were conducted using statistical software SPSS ® 24 (IBM, Armonk, NY, USA).

The research related to human use in this study was performed in compliance with the ethical standards of the 1964 Helsinki Declaration, national regulations, and approved by the Research Ethical Committee, Faculty of Medicine, Universitas Airlangga No. 2000/109/II/2020.

RESULTS

Clinical characteristics

A total of 67 patients diagnosed with testicular GCT were included in this retrospective study. Table 1 showed the summary of clinical characteristics of the patients. The mean age was 28.9 years old, ranging from 8 months to 52 years old. The pathological findings showed that there were 40 patients (59.7%) presented with seminoma, and 27 patients (40.3%) presented with non-seminoma. There were 13 patients (19.4%) with Yolk sac pathology, 5 patients (7.5%) with embryonal carcinoma, and 5 patients (7.5%) with mixed GCT. Death was reported in a total of 9 patients, where 6 cases (9%) were caused by progressive disease and 3 cases (4.5%) were caused by chemotherapy adverse events (Table 1).

Tumor staging

The most frequently reported tumor stage was T2, which occurred in 28 patients (41.8%), followed by T4 in 5 patients (7.4%), and T3 in 22 patients (32.8%). Regarding nodal staging, N3 was the most commonly reported nodal stage with a total of 35 patients (52.2%). Metastatic disease (M1a/M1b) found in 52 patients (77.6%) (Table 1). From tumor marker staging, there were 19 patients (28.4%) with S1, 25 patients (37.3%) with S2, and 14 patients (20.9%) with S3. Based on the 2009 TNM substage classification, there were 8 patients (11.9%) with stage I, 3 patients (4.5%) with stage II, and 56 patients (83.6%) with stage III. According to the age group, seminoma GCT was predominantly presented at the age group of 30 to 39 years (65%), while non-seminoma GCT was primarily found in the age group of 0 to 9 years (40.3%).

Chemotherapy regimens

The most frequently used chemotherapy regimen was four cycles of bleomycin, etoposide, and Cisplatin (BEP), which were administered in 36 patients (53.7%), followed by three cycles of BEP in 22 patients (32.8%) (Table 1). Other chemotherapy regimens used in this study were four cycles of Etoposide and Cisplatin (EP) in 1 patient (1.5%) and one cycle of carboplatin in 1 patient (1.5%) (Table1).

Chemotherapy adverse events

Table 1 displayed the details regarding the adverse events related to chemotherapy. Regarding adverse events evaluation, haematology and digestive systems were the most commonly affected systems occurred in 56 patients (83.6%) and 44 patients (66%), respectively. Neutropenia was the most frequent haematological adverse event in 42 patients (62.7%). In addition, severe nausea and vomiting requiring parenteral nutrition were reported in 4 patients (6%),

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Table 1. Clinical characteristics of the patients with testicular GCT

Variables	n (%)
Age (mean ± SD)	28.9 ± 12.6
T classification	
T1/TX	12 (17.9%)
T2	28 (41.8%)
T3	22 (32.8%)
T4	5 (7.4%)
N classification	
N0/x	15 (22.4%)
N1	7 (10.4%)
N2	10 (14.9%)
N3	35 (52.2%)
M classification	
M0	15 (22.4%)
M1a/M1b	52 (77.6%)
S Classification	
S0	9 (13.4%)
S1	19 (28.4%)
S2	25 (37.3%)
S3	14 (20.9%)
Pathology	
Seminoma	40 (59.7%)
Non-Seminoma	27 (40.3%)
Embryonal Carcinoma	5 (7.5%)
Yolk Sac	13 (19.4%)
Mixed Germ Cell	5 (7.5%)
ICGGGC prognostic factor	
Good	27 (51.9%)
Intermediate	14 (26.9%)
Poor	11 (21.2%)
Pretreatment laboratory parameter	
Neutrophil (mean ± SD)	6.5 ± 3.68
Lymphocyte (mean ± SD)	2. ± 1.2
NLR (mean ± SD)	3.79 ± 2.16
UICC Stage	
IA/B/S	8 (11.9%)
IIC	3 (4.5%)
IIIA/B/C	56 (83.6%)
Chemotherapy regimens	
1 Cycle BEP	7 (11.9%)
3 Cycle BEP	22 (32.8%)
4 Cycle BEP	36 (53.7%)
4 Cycle EP	1 (1.5%)
1 Cycle Carboplatin	1 (1.5%)
Death	
Death due to progressive disease	6 (9%)
Death due to chemotherapy-related adverse events	3 (4.5%)

Clinical response

Table 2 showed the patients' clinical response to the chemotherapy. Based on the ICGGGC prognostic risk classification, 14 patients (58.3%) with good prognostic risk responded completely to the chemotherapy. In the intermediate and poor-risk group,

Clinical response

Table 2 showed the patients' clinical response to the chemotherapy. Based on the ICGGGC prognostic risk classification, 14 patients (58.3%) with good prognostic risk responded completely to the chemotherapy. In the intermediate and poor-risk group, most of the patients had progressive disease, which was occurred in 3 patients (25%) and 5 patients (55.6 %) retrospectively. According to the pathological findings, patients with seminoma GCT mostly had a complete response (54.1%), whereas patients with non-seminoma GCT mostly had progressive disease (47.8%). Furthermore, NLR was observed to be higher in patients with stable disease (6±2.8) and progressive disease (5.46±1.88) compared to patients with complete and partial response (2.54±1.26 and 4.42±2.36, respectively).

Table 2. Frequency of patients with seminoma and non-seminoma GCT according to the age group

	Seminoma	Non-seminoma
Age (year)	n (%)	n (%)
Mean ± SD	34.5 ± 7.37	20.67 ± 14.38
Total	40 (59.7%)	27 (40.3%)
0-9	0 (0%)	9 (33.3%)
10-19	1 (2.5%)	2 (7.4%)
20-29	5 (12.5%)	9 (33.3%)
30-39	26 (65%)	4 (14.8%)
40-49	7 (17.5%)	3 (11.1%)
50-59	1 (2.5%)	0 (0%)

Factors associated with patient's response

The summary of analysis of factors associated with patients' responses was summarized in table 3 and 4.

Table 3. Summary of adverse events related to chemotherapy

Adverse events	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	Total n (%)
Hematological system					
Thrombocytopenia	8 (72.7%)	2 (18.2%)	0 (0%)	1 (9.1%)	11 (16.4%)
Leukopenia	0 (0%)	2 (66.7%)	1 (33.3%)	0 (0%)	3 (4.5%)
Anemia	0 (0%)	9 (90%)	1 (10%)	0 (0%)	10 (14.9%)
Febrile Neutropenia	0 (0%)	0 (0%)	6 (100%)	0 (0%)	6 (9%)
GI system					
Nausea	2 (4.9%)	35 (85.4%)	4 (9.7%)	0 (0%)	41 (61.2%)
Vomiting	1 (6.2%)	11 (68.7%)	4 (25%)	0 (0%)	16 (23.8%)
Mucositis oral	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (1.5%)
Sepsis					5 (7.4%)

Based on the result of bivariate analysis, we found that NLR, age, M staging, S staging, and pathology had a significant association with patient's response ($p < 0.25$), and thus we include those variables in the multiple logistic regression analysis. The multiple logistic regression analysis, we discovered that NLR and S staging were independently associated with the patient's response to chemotherapy (OR 2.14, 95% CI 1.22, 3.78, $p < 0.01$; OR 9.43, 95% CI 1.81, 49.14, $p < 0.01$) (Table 4).

Table 4. Response to chemotherapy

	Complete n (%)	Partial n (%)	Stable n (%)	Progressive n (%)
ICGGGC risk				
Good	14 (58.3%)	6 (25%)	0 (0%)	4 (16.7%)
Intermediate	2 (16.7%)	6 (50%)	1 (8.3%)	3 (25%)
poor	1 (11.1%)	2 (22.2%)	1 (11.1%)	5 (55.6%)
Pathology				
Seminoma	20 (54.1%)	12 (32.4%)	1 (2.7%)	4 (10.8%)
Non-seminoma	8 (34.8%)	3 (13%)	1 (4.3%)	11 (47.8%)
NLR (mean ± SD)	2.54 ± 1.26	4.42 ± 2.36	6 ± 2.83	5.46 ± 1.88

DISCUSSION

The incidence of testicular cancer peaks near birth, followed by a shallow rate before the second peak occurring safter puberty (Nistal et al. 2016). It follows a bimodal distribution with the initial peak happening before the age of four and the second peak occurring in the late 20s and early 30s (Steliarova-Foucher et al. 2017). The mean age of our patients was 29 years old, which was in line with global statistics.

The incidence rate among Asian men were ten times lower than Caucasian population (Park et al. 2018). However, most existing publications are limited to small groups in a few countries, making it difficult to compare the data with worldwide geographic patterns (Kusler & Poynter 2018). In a study which forecasted the future of testicular GCT incidence in the United States, the incidence in the Asian population was expected to rise, even though insignificantly (Ghazarian et al. 2017). Testicular GCTs develop from premalignant intratubular germ cells due to the failure of gonocytes maturation during fetal development. The progression toward invasive GCTS as seminoma or non-seminoma started after puberty (Batool et al. 2019). Seminoma and non-seminoma types make up almost 99% of all testicular GCTs (Farmanfarma et al. 2018).

The pathological findings of the patients showed that 40 patients (59.7%) presented with seminoma, and 27 patients (40.3%) presented with non-seminoma. These findings are similar to the distribution of types reported globally (Kusler and Poynter, 2018). Testicular germ cell tumors are believed to be chemosensitive with a high cure rate, even in a metastatic stage (Semaan et al. 2019).

In recent years, platinum -based chemotherapy has been given to improve the mortality rate of testicular GCT patients, with an overall current cure rate of more than 90% (Baroni et al. 2019). Current clinical data about BEP chemotherapy response in testicular cancer showed that BEP is the most effective combination regimen in treating disseminated non-seminomatous germ cell cancer. In this study, one patient did not receive Bleomycin because of poor pulmonary function.

Based on the recommendation reported by the European Association of Urology (EAU) guideline, one course of BEP regiment could be used in a patient who was unwilling to undergo surveillance, where efficacy had proven to be superior compared to RPLND. Patients undergoing surveillance could also undergo chemotherapy (Albers et al. 2015). The expected response rate was more than 90% in patients with a good prognosis, with few patients relapsing. Most patients in this study were given BEP, except for one patient who received Carboplatin. A total of 14 patients (58.3%) with reasonable prognostic risk had a complete response to the chemotherapy. In the intermediate and poor-risk group, most patients had progressive disease. Patients with seminoma GCT mostly had a complete response, whereas patients with non-seminoma GCT mostly had progressive disease. Although BEP chemotherapy produced excellent outcomes, it was associated with more toxicity, especially in pulmonary toxicity (den Hollander et al. 2016).

There was a 5% toxic-related death, sepsis or bleomycin-induced pneumonitis (Efstathiou & Logothetis 2006). There was a risk of leukemia associated with the amount of dose of chemotherapy (Howard et al. 2008). Cisplatin was associated with acute myeloid leukemia in a dose-dependent relationship. Hypogonadism and metabolic syndrome were also frequent in testicular cancer patients undergoing chemotherapy (Bogefors et al. 2017). A cardiovascular event incidence was also higher than the normal population (Lauritsen et al. 2020). In this study, hematological manifestation was the most common. A severe complication, such as sepsis, occurred in five patients, where three died due to septic shock and severe neutropenia.

Regarding response and prognosis, inflammation plays a role in the progressivity and prognosis of GCTs. One of the markers was NLR. Inflammatory cells produce mediators and cytokines that can induce or promote angiogenesis, tumor growth, invasion, and metastasis (Karakaya et al. 2021). Systemic inflammation has an essential role in all tumorigenesis stages. It may induce the process via genetic mutations and genomic instability. Inflammation can also activate tissue repair, inducing the proliferation of premalignant cells (Grivennikov et al. 2010).

The current hypothesis states that the synthesis of inflammatory cytokines was affected by the tumor micro-environment, causing acute reactive changes in the neutrophil count (Tan et al. 2019, Ilktac et al. 2020). Both neutrophils and lymphocytes are essential inflammatory mediators in many cancer types. Several studies suggested that neutrophil count supports tumor growth, while suppressing anti-tumor response (Fridlender & Albelda 2012). The activation of neutrophils might suppress lymphocyte function, causing immunosuppression and releasing enzymes with low anti-tumor activity (Gooden et al. 2011).

The parameter has been reported in other malignancies, such as breast cancer, in which several studies have used it to predict chemotherapy success (Chae et al. 2018). A study conducted by Hirahara et al. on gastric cancer patients concluded that the NLR above the cut-off point of 2.46 is associated with higher disease progressivity (Hirahara et al. 2019). A meta-analysis study concluded that NLR below the cut-off point of a specific population of patients was associated with a higher chance of complete response in solid tumor patients receiving chemotherapy (Li et al. 2018). Several studies are evaluating its role in germ cell tumors.

In this study, we observed a higher level of NLR in patients with stable disease (6±2.8) and progressive disease (5.46±1.88) compared to patients with complete and partial response (2.54±1.26 and 4.42±2.36, respectively). After grouping the patients according to their response to the chemotherapy (complete and non-complete) in the bivariate analysis, we found that NLR, age, M staging, S staging, and tumor pathology had a significant association with the patient's response. Furthermore, from the result of the multiple logistic regression, we discovered that NLR was independently associated with the patient's response to the chemotherapy (OR 2.14, 95% CI 1.22, 3.78, p <0.01). A similar finding also reported a correlation between high NLR value and low chemotherapy response rate (Karakaya et al. 2021). Survival parameters were reported that NLR was associated with progression-free survival (PFS) and overall survival (OS) (Ribnikar et al. 2021). Even though this study showed that NLR seemed to be higher in patients with stable and progressive disease than patients with partial and complete response, a definitive conclusion still could not be made until a prospective cohort prognostic study with larger sample size is performed.

Strength and limitatioan

This study had several limitations. It was conducted retrospectively in one center with a moderate sample size. Multiple future studies should be conducted in centers all over Indonesia to fully understand the demographic and clinical characteristics, and the chemotherapy response of Indonesian patients with testicular GCTs. A cohort analytical study analyzing the prognostic value of risk factors can be performed in a multicenter study with larger sample size.

CONCLUSION

The clinical characteristics and response of testicular GCT patients among Indonesian men showed similarity with current literature representing worldwide data. However, more extensive multicenter study is required to grasp the pattern of characteristics in Indonesian patients. The NLR results in this study indicated a potential biomarker as a poor prognostic factor in testicular GCT. However, further studies were required to fully determine its value.

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Conflict of interest

None.

Funding disclosure

None.

Author contribution

HMS and LH contributed conseptual, study design, collected and analysis data. HMS was write the manuscript. LH was checking grammar and validation of all manuscript data.

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Original Article

HEMOGLOBIN A1C (HBA1C) IS STRONGLY CORRELATED WITH MEAN CORPUSCULAR VOLUME AMONG TYPE 2 DIABETES MELLITUS (T2DM) PATIENTS ADMITTED TO A TERTIARY HOSPITAL IN EAST JAVA, INDONESIA

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ABSTRACT

HbA1c showed the average level of blood sugar in the recent 2-3 months. This parameter can be used to help physicians to diagnose T2DM and to plan appropriate treatment. Meanwhile, the mean corpuscular volume (MCV) is established from the erythrocytes levels as one of the blood corpuscles, to which hemoglobin is bound. We hypothesized that MCV was correlated to the HbA1c levels and could be an indicator of blood sugar levels in adult T2DM patients. A retrospective cross-sectional study based on the medical record of patients admitted to the Outpatient Section of Department of Internal Medicine, Dr. Soetomo General Academic Hospital, Indonesia from January to December 2019 was done. Patients under 18-years old and incomplete medical record data were excluded. Adult patients diagnosed with T2DM and received initial treatment in this section were included (n=1.688). Data were analyzed using a correlation test in SPSS 17.0 (USA), a p-value less than 0.05 was considered significant. We found a significant negative correlation between HbA1c and MCV levels in these patients (r= -0.312; p<0.001). MCV levels showed a paradoxical pattern against the blood glucose levels in T2DM and could serve as the health indicator in these patients.

Keywords: *Diabetes; hemoglobin; HbA1c; MCV; health risk*

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Highlights:

1. Moderate negative significant correlation between HbA1c and MCV levels in T2DM was yet reported widely.
2. MCV levels could mirror health indicator in adult T2DM patients.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia caused by abnormalities in insulin secretion, insulin action, or both (PERKENI 2019). The prevalence is about 422 million amongst adult diabetes patients worldwide (World Health Organization 2021), while the prevalence of T2DM in 2018 in Indonesia was approximately 20.4 million (8.5%). This has increased from 2013 which was around 6.9%. A higher prevalence was reported in women than men (12.7% vs. 9.0%) (PERKENI 2019).

HbA1c is one of the laboratory tests to determine the diagnosis of T2DM, that is if the HbA1c level is >6.5%, someone is considered to have diabetes. Complications in T2DM are associated with increased risk factors for ischemic heart disease and impaired renal function, with abnormalities detected in complete blood cells laboratory results, including the hemoglobin and MCV levels (Alamri et al. 2019). It has been reported that HbA1c as the chronic indicator of blood glucose level in T2DM patients was closely correlated with the MCV levels (Rodriguez-Segade et al. 2016). Abnormality in MCV level is due to

hyperglycemia in T2DM patients resulting in relative depletion, hence affecting the oxygen transfer via hemoglobin in the red blood cells (Maner & Moosavi 2021, Wang et al. 2021). However, in our region, whether the MCV level reflects the HbA1c in T2DM has yet largely been determined, thus aimed in the current study. If MCV is related to the levels of HbA1c, this parameter can serve as the potential biomarker for detecting blood glucose levels, specifically in T2DM cases that would help physicians to be more aware of the health status and understand the success in T2DM patients' treatment.

MATERIALS AND METHODS

This study has been granted ethical clearance by the health research ethics committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, No.:0195/LOE/301.4.2/XI/2020. We conducted a retrospective cross-sectional study from the medical record of adult patients aged more than 18 years old admitted to the Outpatient Section of the Department of Internal Medicine, Dr. Soetomo General Academic Hospital, from January to December 2019. Data recorded including diagnosis of T2DM, HbA1c level, MCV level, all taken after first treatment by the physician according to the protocols in this institution. All data were analyzed using normality and homogeneity test and Spearman correlation test to seek potential correlation between HbA1c and MCV levels in these patients; the p-values less than 0.05 were considered statistically significant. The correlation strength is defined as very strong if $r > 0.75$; strong 0.5 to 0.75; moderate 0.25-0.5; and weak < 0.25 (SPSS 17.0, USA).

RESULTS

Of the 1.688 medical records, 717 (42.5%) were males and 971 (57.5%) were females. The mean of HbA1c levels of all patients was 8.15% ± 1.89%. The mean of MCV levels of all patients was 84.39 fl ± 6.87 fl. The mean HbA1c level in female patients was 7.93% ± 1.82%; while in the male patients was 8.30% ± 1.92%. The mean MCV level in female patients was 86.23 fl ± 6,09 fl; while in the male patients was 90.09 fl ± 13.70 fl.

There were significant differences between female and male patients in the HbA1c levels ($p=0.002$). There were no significant differences between female and male patients in regards of the MCV levels ($p=0.144$). There was a significant moderate correlation between HbA1c and MCV levels in all patients ($r= -0.312$; $p<0.001$) (Table 1).

DISCUSSION

HbA1c mirrors the average level of blood sugar in approximately 2-3 months (Wang et al., 2021). This test can be used to help physicians to diagnose T2DM, thus planning the appropriate statement. It was reported that the HbA1c level in non-T2DM people is approximately 4-5.6%. Increased HbA1c level means pre-diabetic condition; higher than 6.5% is generally defined as diabetes. The management in diabetes patients targets HbA1c level to be less than 7%. Untreated diabetes patients in a while might be detected by increased HbA1c above 8%.

Combination of proper diet, exercise and meditation would bring HbA1c level into normal, the doctors usually ask the patient to test their HbA1c level every 3 months to make sure it is under controlled. However, if at least tested 2 times a year could be considered sufficient (Eyth & Naik 2021, PERKENI 2019). Several comorbidities, including anemia, could result in misinterpretation of HbA1c level. Supplements including vitamin C and E, kidney and liver diseases, dyslipidemia could also affect the hemoglobin levels thus misleading the result of HbA1c test (Eyth & Naik 2021).

HbA1c represents a glycated hemoglobin or glycosylated hemoglobin, where glucose is bound to the erythrocytes. In the erythrocytes, hemoglobin is the protein carrying the oxygen, whilst HbA1c levels reflect the percentage of all hemoglobin that are bound to the glucose. HbA1c showed the last 2-3 months blood glucose level average due to the erythrocyte life cycles in this period (Wang et al. 2021).

On the other hand, mean corpuscular volume (MCV) showed the average volume of red blood corpuscles, which mean multiplication of blood volume by the proportion of cellular components in the blood and divided by the number of erythrocytes in that volume. There are 3 types of corpuscles in human blood, which are erythrocytes, leukocytes and platelets. Abnormal MCV might indicate impaired corpuscle morphology and or count including the red blood cell. Impairment could lead to abnormal oxygen capacity and transfer into the tissue organs. The normal range of MCV in the adults is approximately between 80-100 fl. If a person has less than 80 fl, someone is likely suffering from the microcytic anemia. However, if the MCV levels are higher than 100 fl, the macrocytic anemia is determined (Maner & Moosavi 2021).

Certain conditions must be taken into consideration on interpreting the MCV levels. In patients with liver disease and folate deficiency, the MCV might be

Table 1. Statistical analysis between HbA1c and MCV levels in all included T2DM patients

	HbA1c level (%)				MCV level (fl)				#r	#p
	Male	Female	p (Mann-Whitney test)	Total	Male	Female	p (Mann-Whitney test)	Total		
Mean (±SD)	7.93 ± 1.82	8.30 ± 1.92	0.002	8.15 ± 1.90	90.09 ± 13.70	86.23 ± 6.09	0.144	84.39 ± 6.87	-0.312	<0.001*

#Spearman's correlation test
*: "correlation is significant at the 0,05 level (2-tailed)"

increasing. Increased MCV can also be used to monitor alcohol related disease, with macrocytosis detected. Macrocytosis itself generally asymptomatic, several drugs that may produce macrocytosis including methotrexate, antiretroviral agents, valproic acid, phenytoin, zidovudine, azathioprine, and hydroxyurea (Nagao & Hirokawa 2017). Previous study reported that stress can significantly increase the hemoglobin and MCV levels and impaired tissue oxygenation (Maes et al. 1998). However, this could be due to more serious reasons i.e. pernicious anemia; whilst another study reported that MCV more than 106 fl was correlated with the increased risk of esophageal squamous cell carcinoma amongst Japanese alcoholic man (Yokoyama 2003). On the other hand, in low MCV levels, the erythrocytes are found to be smaller than normal, this might be due to iron deficiency correlated with poor dietary intake of iron, or bleeding, including menstrual bleeding, and gastrointestinal bleeding. The treatment of microcytic anemia with low MCV level includes iron and vitamin C supplements. Other pathologies such as thalassemia and hypothyroidism might cause low MCV levels (Maner & Moosavi 2021).

In T2DM with very high glucose levels, increased MCV can be detected. The correction can be conducted by pre-dilution of the blood in isotonic medium, thus the hyperosmolar state produced by high glucose level in the cell might result in rapid diffusion of water intracellular in the counter. This process is temperature dependent and could be rapidly reversible (Morse et al. 1981). It was reported that HbA1c was significantly and independently associated with MCV levels among 50-year-old females (Simmons & Hlaing 2014). Another study by Rashed et al. (2020) found that HbA1c level of 202 patients measured by NycoCard reader II analyzer has significant negative correlation with the MCV level, the mean MCV was significantly higher in non-diabetic patient, when compared with pre-diabetic and diabetic patient. The red blood cell life span in hyperglycemia decreased compared with in the normoglycemia (Rio et al. 2016). The glycation synthesis of the terminal unit of the β-chain in hemoglobin is determined by the plasma glucose level and has been used to evaluate the level of metabolic control and the development of complications in T2DM patients, thus the quality of diabetic management (Leow 2016).

Strength and limitation

The study uses a large sample size of 1,688 patients, which increases the generalizability of the findings.The study focuses on a relevant topic, which has the potential to improve the diagnosis and treatment of T2DM.The study uses a retrospective cross-sectional design, which is appropriate for exploring associations between variables and can provide useful information for generating hypotheses.The study design is limited in that it can only establish associations between variables and cannot determine causality.The study only examines the correlation between MCV levels and HbA1c and does not consider other factors that may influence blood sugar levels in T2DM patients, such as diet, exercise, and medication.

CONCLUSION

HbA1c and MCV levels are determined by blood glucose level. This study shows significant negative correlation between HbA1c and MCV level in adult T2DM patients admitted in our institution.

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Conflict of interest

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Author contribution

RRM, JHP, and YS contributed conseptual, study design, analysis data. YS was write the manuscript. VPK was final validation.

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Original Article

PROSTATE-SPECIFIC ANTIGEN AND TIME TO PSA NADIR AS PROGNOSTIC SIGNIFICANCE IN CASTRATION-RESISTANT PROSTATE CANCER

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ABSTRACT

A high mortality rate was often found in castration-resistant prostate cancer (CRPC). This study was to assess the PSA level and time to PSA nadir as a prognostic tool for survival in CRPC patients. Several factors are considered to be useful as prognostic markers in CRPC patients. This study was a descriptive study assessing the survival rate in castration-resistant prostate cancer. Evaluation data included sex, age, initial PSA level, final PSA level, time to PSA nadir (TTN), time to CRPC progression (TTC), and survival status. A total of 24 patients with CRPC were evaluated in this study. Our result revealed that there was a significant difference found in the initial PSA level between survivors (445.7 + 165.6 ng/mL) and non-survivor (200.7 + 144.9 ng/mL). There were no significant differences found in PSA nadir level, TTN, and TTC between survivor and non-survivor groups. However, there was a significant correlation between time to PSA nadir and time to CRPC progression. This study revealed that there was an association between initial PSA level on the survival rate of CRPC patients. Initial PSA level could be used to predict survival prognosis in CRPC patients.

Keywords: *Prostate-specific antigen; PSA nadir; prostate cancer; castration-resistant prostate cancer*

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Highlights:

1. Assessing the survival rate in castration-resistant prostate cancer was descriptived.
2. PSA level between survivor and non survivor was significant but not for PSA nadir level, TTN and TTC.
3. Initial PSA level can predict survival prognosis rate of castration-resistant prostate cancer patients.

INTRODUCTION

Of all-male cancers worldwide, prostate cancer accounted for 15% and became the second most common malignancy in men (Barsouk et al. 2020). The study showed that 1 in 25 men will be most likely to develop prostate cancer in their lifetime (Bray et al. 2018). The gold standard therapy for metastatic prostate cancer was primary androgen deprivation therapy (ADT). Most patients will experience a substantial decline in PSA which leads to undetectable PSA for years. However, even after ADT, PSA level may fail to decrease and disease progression may occur in some cases (Tomioka et al. 2014). This progression is known as castrate-resistant prostate cancer (CRPC). Few are currently understood about the factors influencing the survival of CRPC patients. The variability in the clinical course of CRPC led to the utilization of several prognostic factors regarding their roles influencing the treatment strategy and its capability to predict the response of therapy. One known factor which could be evaluated as prognostic value is prostate specific antigen (PSA) level, even though using PSA as a single predictor for prognosis in prostate cancer patients may be unreliable (Ørsted et al. 2012). Recent studies have reported the utilization of initial PSA level, time to PSA nadir (TTN), PSA nadir level, and time to CRPC progression (TTC) among other parameters for predicting the prognosis of CRPC patients (Hamano et al. 2019). Several studies have suggested that nadir PSA level was the most significant predictor of CRPC progression. PSA rising after the

nadir value after PADT (primary androgen deprivation therapy) may reveal the sign of CRPC.

The association between time to PSA nadir (TTN) with progression, cancer-specific death, and all-cause mortality was demonstrated in a previous study (Choueiri et al. 2009). A rapid reduction of PSA level after ADT might be due to ablation of androgen receptor function. Since androgen receptor plays a role as a tumor suppressor in prostate cancer, rapid suppression of androgen receptor during ADT may lead to negative effect on a disease progression (Huang et al. 2011). Another study also reported that longer time to CRPC progression may be correlated with improved overall survival (Frees et al. 2018). Therefore, this study aimed to evaluate PSA level and time to PSA nadir (TTN) as a prognostic marker for survival in CRPC patients.

MATERIALS AND METHODS

This is a descriptive study evaluating the characteristics of prostate cancer patients based on the medical record date from Dr. Soetomo General Academic hospital from January 2013 to December 2020. This study has been approved by the ethical committee of Dr. Soetomo General Academic Hospital under a decree number 0392/129/XI/2020. On prostate cancer patients treated with castration-resistant progression were included in this study. Data evaluation consisted of sex, age, initial PSA level, final PSA level, time to PSA nadir (TTN), time to CRPC progression (TTC), and survival status. The progression of CRPC is defined as a rising PSA level and or radiographic progression evidence despite medical or surgical castration (Lowrance et al., 2018). Initial PSA level of the patients is defined as PSA level at the time of admission, whereas final PSA nadir level is defined as the lowest level after castration. Time to PSA nadir is defined as the time from castration until the lowest level of PSA is reached. The time from PSA nadir level to the development of castration resistance is defined as time to CRPC. All variables are presented descriptively in graphs.

The normality of distribution was performed with a Shapiro-Wilk test. If the data was normally distributed, an Independent T-test was performed to evaluate the differences for numerical variables between the surviving and non-surviving groups of patients, otherwise a Mann-Whitney U test would be used. To evaluate the correlation between numeric variables, we used Pearson correlation test. P value of less than 0.05 was considered to be significant.

RESULTS

Baseline characteristics

In this study, 24 patients with CRPC were included. The average age of the samples was 65.54 ±7.5 years old. The patients' initial PSA was 388.57±596.7 ng/mL. Four patients were performed medical castration, while 20 patients were performed surgical castration. It took approximately 308.4 ± 293.7 days for PSA level to reach PSA nadir. The lowest PSA level was 46.4±112.5 ng/mL on average. The average time for the patients to develop CRPC was 554.1±437.1 days. Baseline characteristic of the patient was shown in Table 1.

Table 1. Baseline characteristics of the patients

Variables (mean)	Value
Age	65.54
Initial PSA level (ng/ml)	388.57 ± 596.7
Castration (n)	
Medical castration	4
Surgical castration	20
PSA nadir (ng/ml)	46.4 ± 112.5
Time to PSA nadir/TTN (days)	308.4 ± 293.7
Time to CRPC/TTC (days)	554.1 ± 437.1

Initial PSA and patient survival

There were seven patients who died and 17 patients who survived until the last period of observation. The average initial PSA level of surviving patients was 445.7+165.6 ng/mL, whereas the PSA level for patients who did not survive was 200.7+144.9 ng/mL. Because the Shapiro-Wilks test result suggested that the data had a normal distribution ($p>0.05$), an independent T-test was used for a comparative analysis. As indicated in Figure 1, there was a significant difference in initial PSA level between the groups ($p<0.05$) as shown in Table 2.

Table 2. The association between initial PSA level, PSA nadir, time to PSA nadir level and time to CRPC progression to patient survival

Variables	Survivor	Non-survivor	p-value
Initial PSA level	445.7 ±165.6	200.7 ±144.9	< 0.05
PSA nadir	42.8 ±131.9	42.7 ± 48.7	> 0.05
Time to PSA nadir	318.5 ±176.9	284.1 ±510.5	> 0.05
Time to CRPC progression	598.9 ±431.1	445.2 ±499.2	> 0.05

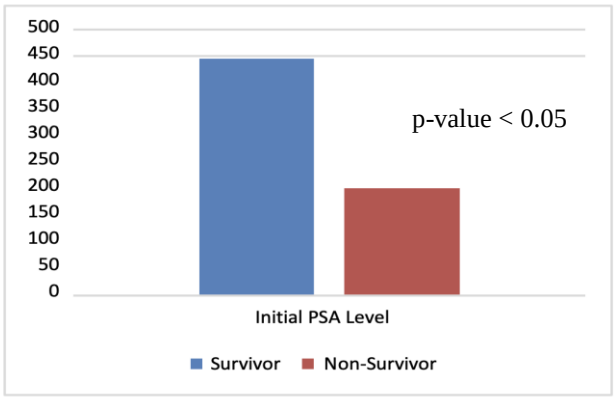

Figure 1. Initial PSA level differences between surviving and non-surviving patients

PSA Nadir and patient survival

The average PSA nadir level among patients who survived was 42.8+131.9 ng/dL, while the average PSA nadir level among patients who died was 42.7+48.7 ng/dL. To compare both groups, we used Mann-Whitney test, due to abnormal data distribution ($p<0.05$). The difference between both groups in Figure 2 was insignificant ($p>0.05$) as shown in Table 2.

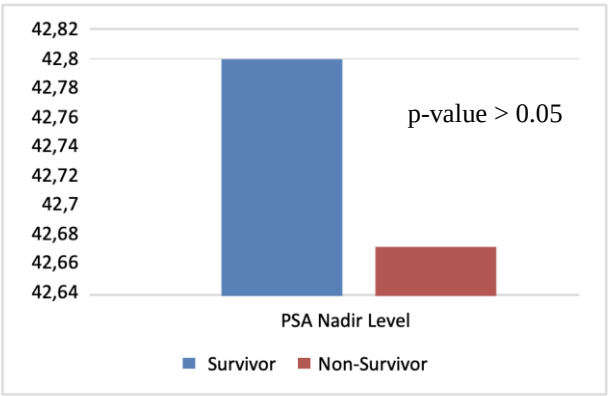

Figure 2. PSA Nadir level differences between surviving and non-surviving patients

TTN and patient survival

The average TTN of the surviving patients was 318.5+176.9 day, whereas the TTN of patients who died was 284.1+510.5 days. Due to abnormal distribution of the data, Mann-Whitney test was used for comparison ($p<0.05$). As indicated in Figure 3, the study revealed no statistically significant difference between two groups ($p>0.05$) as shown in Table 2.

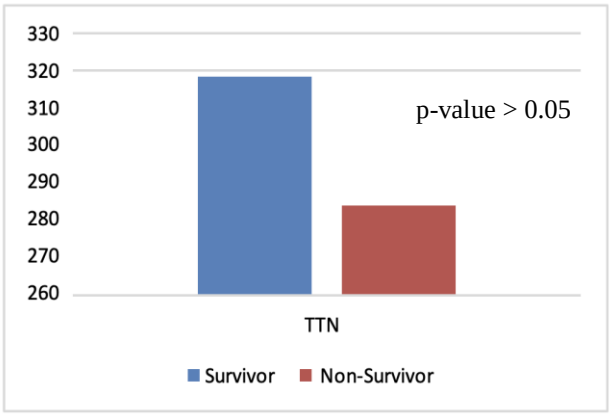

Figure 3. Time to PSA Nadir level difference between surviving and non-surviving patients

TTC and patient survival

The average TTC of patients who survived was 598.9 + 431.1 days, while the TTC of patients who did not survive was 445.2+499.2 days. Mann-Whitney test showed an insignificant difference between the two groups shown in figure 4 ($p<0.095$) as shown in Table 2.

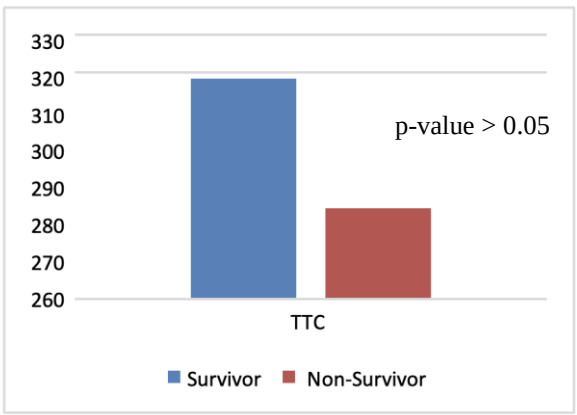

Figure 4. Time to castration-resistant progression between surviving and non-surviving patients

TTN and TTC

Correlation test analysis was performed using Pearson Correlation test to determine the correlation between TTN and TTC. Our result revealed that there was significant positive correlation ($p<0.05$) between TTN and TTC with correlation coefficient of 0.737 as described in Figure 5.

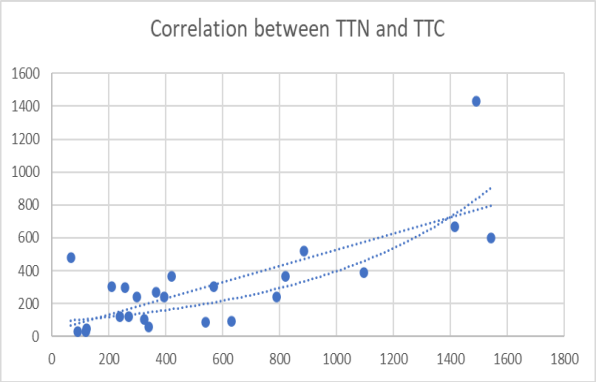

Figure 5. Correlation between TTN and TTC

DISCUSSION

PSA tests are used on a regular basis to screen prostate cancer and monitor progression (Ilic et al. 2018). PSA monitoring is important for evaluating treatment response during androgen deprivation therapy (ADT). In the first month following ADT treatment, most patients had a decrease in PSA levels (Sasaki & Sugimura 2018). In this study, there were 24 evaluated patients with CRPC. Our results found that initial PSA level was significantly associated with patient survival. Moreover, time to PSA nadir (TTN) and time to CRPC progression (TTC) was also significantly correlated. However, there was no significant association between nadir PSA level, time to PSA nadir (TTN) and time to CPRC progression (TTC) to patient survival.

The majority of patients with high initial PSA level reflected severity of tumor characteristics or an asymptomatic tumor for a long period of time, indicating the possibility that the patient is neglectful of his condition (Kan et al. 2017). A high PSA level also indicates a high androgen receptor activity of prostate cancer cells (Iwamoto et al. 2019). Previous studies also highlighted the mortality risk of a high PSA level. On the contrary, patients with lower PSA levels in this study had significantly higher mortality rate compared to patients with relatively higher PSA levels ($p<0.05$). The difference in findings was possible possibly due to the bias of PSA measurement and age variation among patients. The evaluation of age difference is often not assessed in measuring initial PSA (Heidegger et al. 2015). The sensitivity and specificity of PSA measurement wass low due to several factors affecting PSA level, such as catheterization, post-coitus, benign prostatic enlargement (BPE), and prostate infection (McAninch & Lue 2020).

Our result also found that there was no correlation between TTN and survival ($p>0.05$). Patients with TTN of less than 9 months had a considerably greater overall survival rate than those with TTN of more than 9 months, according to the study. A previous large scale retrospective study analyzing 89 patients conducted between 2000 and 2009 found a significant association between TTN and survival. Also, the study also indicated that patients with TTN of less than 9 months had a considerably better overall survival rate than those with TTN of more than 9 months as well as discovering that a PSA nadir level of less than 0.2 ng/mL was associated with a better prognosis (Sasaki et al. 2011). Number of studies suggested that TTN and survival rate may be due to nature of some prostate cancer cells which can adapt to castration by utilizing intracrine androgens. During castration, androgen-sensitive cells would perish, while cells which can produce intracrine androgen (Sasaki & Sugimura 2018).

Our finding also revealed that the TTC was not associated with the patients' survival rate. These findings were different compared to a previous retrospective study evaluating 287 patients from 1996 to 2009, which reported that TTC was an independent factor to predict overall survival and progression-free survival. The study claimed that TTC less than two years was associated with a worse prognosis (Frees et al. 2018). Another retrospective study evaluating 289 patients from 2008 to 2015 reported a positive association between TTC and survival. Interestingly, the study also reported a positive association between hormone sensitive prostate cancer (HSPC) and patient survival (Bournakis et al. 2011). The differences of the findings in this study compared to previous studies may be due to the small number of samples in this study. Several studies suggested that a low TTC was due to PSA volume and PSA doubling time difference (Iwamoto et al. 2019).

Finally, in this study, the positive significant correlation between TTN and TTC highlighted intriguing implications. This finding was according to previous study which reported that the ability to reach an undetectable PSA level such as nadir was the most significant predictor for the time to CRPC progression in metastatic advanced prostate cancer (Benaim et al. 2002). Previous studies also stated that patients with short TTN were faster to develop castration-resistant (Hamano et al. 2019). The oncogene retinoblastoma protein (pRB) is reduced during castration level, resulting in a reduction of cyclin dependent kinase (CDK).

The prostatic cancer cell replication is halted due to this reduction. In a terminal proliferation phase, there are two possibilities for prostatic cancer cells, apoptosis or continually producing intracranial testosterone at a certain level of castration (Sasaki & Sugimura 2018). The mechanism of dependent androgen receptors has a role in castration-resistant progression. In some cases, androgen is still available at a low concentration even though the ADT has been given. This condition could lead to an adaptation of prostate cancer cells by amplification and an increase of AR expression via a mutation.

The amplification and mutation of AR involve several co-activators and co-repressors. Several studies reported the increase of FKBP51 co-activator in castrated rats. Co-repressor proteins are lower in CRPC patients. Based on the mechanism, several studies concluded that castration which leads to a short TTN would increase the activity of co-activators, while decreasing the activity of co-repressors, inducing the amplification and mutation of AR (Choueiri et al. 2009).

Strength and limitation

This study was limited due to its retrospective design and small sample size. The patients' follow-up period patients could also be extended to assess additional factors that might impact survival rate. The diagnostic modality used to evaluate metastasis was also limited in this study.

CONCLUSION

The initial PSA level differed significantly between survivors and non-survivors. However, there were no significant variations in PSA nadir level, time to PSA nadir, or time to CRPC progression. Our finding also revealed that there was an association between the time to PSA nadir and CRPC progression.

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Conflict of interest

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Author contribution

FHP and WD-conseptual, study design, collected data, and analysis data. FHD-write and revised the manuscript. DMS-final validation and grammar check.

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Case Report

RARE DIAGNOSIS OF A PROLIFERATING PILAR TUMOUR IN A FACIAL HAIRLINE CYST

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ABSTRACT

Sebaceous cyst, also known as an epidermoid cyst, is a subepidermal nodule filled with keratin and it is a benign capsulated tumor. It is often located on the scalp region, face, neck, and trunk; but can be found elsewhere such as the scrotum, genitalia, fingers, and buccal mucosa. Proliferating Pilar Tumors (PPT) are rare tumors. It is derived from the external root sheath of the hair follicle. These tumors are like irregular subcutaneous nodules and often appear on the scalp. This case report was about a 59 years old woman who came to the hospital following excision of a frontal lump elsewhere, with a sebaceous cyst as the initial diagnosis. From the histopathologic examination, grossly there was a whitish and greyish lump with a soft outer surface. Microscopically, there were malignancy signs with areas with keratinization. The tumor formed a solid pattern of enlarged cells with moderate to marked nuclear pleomorphism with vesicular nuclei, prominent nucleoli, and abundant pale eosinophilic to clear cytoplasm. There was also much free keratinous debris noted and numerous foci of calcification identified within the tumor. Mitotic figures with abnormal forms were frequently seen. The final diagnosis after the histopathological examination was Proliferating Pilar Tumour with focal malignancies. In conclusion, facial hairline tumor differentially diagnosed as a sebaceous cyst turned out to be a rare Proliferating Pilar Tumor (PPT). Following histopathological confirmation, the patient was referred for further management by a specialist team.

Keywords: *Sebaceous cyst; proliferating pilar tumor; rare tumor; disease*

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Highlights:

1. Sebaceous cyst or epidermoid cyst is a benign capsulated tumour on the scalp region, face, neck, and trunk that subepidermal nodule filled with keratin.
2. Proliferating Pilar Tumours (PPT) are rare tumours was found in hair follicle.
3. Sebaceous cyst diagnose on facial hairline tumour turned out to be a rare Proliferating Pilar Tumour (PPT).

INTRODUCTION

Sebaceous cyst or *epidermoid cyst* is a benign cyst derived from infundibulum epithelium from the hair follicle. This cyst can grow in every part of the body, grow slowly, and remain present for years. To diagnose this cyst, it needs a histopathological examination. To treat these patients, doctors may do observations, complete excision or incision, drainage of infected cyst, and steroid injection (Naftali et al. 2018). This cyst can happen at any age, from birth until 72 years of age. However, it often happens between 15 years and 35 years (Puranik et al. 2016). Malignancy transformation is so rare, but there is a possibility of it. The malignancies of this cyst can be squamous cell carcinoma, basal cell carcinoma, and *merkel* cell carcinoma (Wollina et al. 2018).

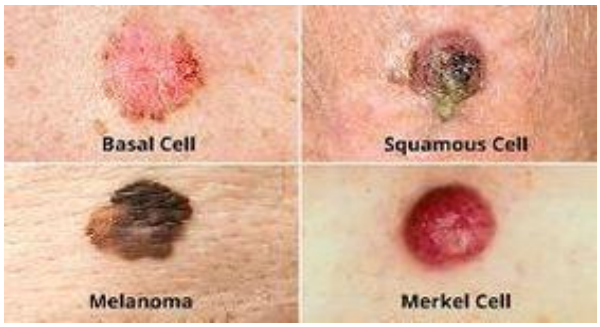

Figure 1. Malignancies of *Sebaceous cyst*

Proliferating Pilar Tumour (PPT) is a very rare tumour, and there are less than 100 cases reported. This tumour can be classified as benign, low- and high-grade malignant tumour because of their different significant biologic activities (Gulati et al. 2011). Because of the rarity of these cases, there are no guidelines available for the management of these tumours. The standard treatment has been still wide local excision (Siddha et al. 2007). Here, this case was initially diagnosed by *sebaceous cyst*, but after the first excision and biopsy, the histopathological examination showed that it was a proliferating pilar tumour with focal malignancies. This will be treated again with re-excision of residual tissue in the area that was previously operated.

CASE REPORT

Female patient, 59 years old, attended Universiti Kebangsaan Malaysia Hospital referral by other hospital. A small lump was found 1.5 cm in diameter in frontal region of the facial hairline. Clinical examination showed a fixed lesion, well-defined and soft on palpation. No cervical lymph nodes palpable (group I – V and facial/ parotid/ occipital all negative). The patient admitted that there was no pain felt in the lump as well. The patient denied having suffered any trauma or having undergone any surgical procedure in that region. She said that the lesion began to develop like 20 years ago but was slow in progression and just decided to go to an expert recently, because she thought that it might be dangerous and this had no symptoms either. The patient had bronchial asthma in her medical history. She had undergone an excision biopsy for the lump in the previous hospital. After the excision, there was a scar in her frontal region 2 x 0.5 cm in size, 8 cm above the right brow, and not palpable.

The histopathology examination received grossly a piece of whitish grayish lump weighing 1.0 g measuring 17 x 16 x 9 mm. The outer surface was smooth. Cut section showed a homogenous whitish yellowish cut surface. Sections microscopically showed a well-circumscribed mass surrounded by thin fibrous capsule composed of islands and lobular proliferation of squamous epithelial cells in areas showing microcystic formation exhibiting glassy eosinophilic cytomorphology. Areas with keratinization were noted. The basement membrane appeared hyalinized and thickened. In areas, the tumor formed a solid pattern having enlarged cells with moderate to marked nuclear pleomorphism with vesicular nuclei, prominent nucleoli and abundant pale eosinophilic to clear cytoplasm. There were also many free *keratinous debris* noted. Numerous *foci* of calcification were identified within the tumor. Mitotic figures were frequently seen; showing abnormal form. No necrosis present. It was diagnosed as a frontal *sebaceous cyst* at first, but after histopathology examination, it was diagnosed as a proliferating pilar tumor with focal malignant features. Because of this histopathology examination, the patient was referred to Universiti Kebangsaan Malaysia Medical Centre for the next treatment.

When operated in another hospital before coming to Universiti Kebangsaan Malaysia Medical Centre, there was no marginal resection when operated before, because it was possibly just diagnosed as a benign *sebaceous cyst*, so that the next treatment was an additional surgery, and it was a re-excision of the residual tissue of the area that has been previously operated to prevent the presence of the residue of cancer cells in that area, because the patient had been diagnosed by malignant tumor.

DISCUSSION

Epidermoid Cyst often occurs in the head and neck region, for example the scalp (34%) and neck (18%) often occurred in the areas (Nicollas et al. 2000). This benign cyst grows very slowly and usually occurs without any symptoms, but inflammation may happen and cause pain (Nicollas et al. 2000, Ohta et al. 2012, Weedon & Strutton 2010). This cyst was so rare to become a malignancy, but there were some cases of malignancies, such as Basal Cell Carcinoma, Bowen disease, Squamous Cell Carcinoma, and Mycosis Fungoides (Debaize et al. 2002). This cyst can happen in any age and often in the third and fourth decade of life (Sunil et al. 2014).

There was an English literature that showed a case of malignancy in 1968, when McDonald, in his analysis of 637 epidermal and sebaceous cysts, found malignancy only in seven cases (1.1%). There were just eight cases of basal cell carcinomas and only one was a squamous cell carcinoma. There was no metastasis. The treatment was local excision and it was satisfactory (Arianayagam & Javalakshmi 1987).

Proliferating Pilar Tumor (PPT) was first described by Wilson-Jones in 1966 named as “proliferating epidermoid cyst". After that, there were so many names to name this disease. There were proliferating trichilemmal cyst, hydatidiform keratinous cyst, giant hair matrix tumor, invasive hair matrix tumor, and trich chlamydia carcinoma. It showed that there were many interpretations about the biological activity of this tumor (Javid et al. 2020).

Proliferating Pilar Tumor (PPT) often occurs in elderly woman patients. There were 76 cases that divided PPT into three groups based on the degree of stromal invasion and the level of cytological atypia–benign, low- and high-grade malignant. A study indicated that there were 20 cases of low-grade malignant tumor and occurred in age 64.1 with men to female ratio of 1:2. The tumor location often occurred in the head and neck in 80% cases. It also often happens in the scalp (Network 2004). Most of the cases had benign lesions before it was operated (Gulati et al. 2011). Proliferating Pilar Tumors usually attack patients with excess hair than patients with bald scalp. These pilar tumors grew from the increasing of epithelial proliferation within pilar or *sebaceous cyst*. Patients often gave a history that they have a long remaining cyst in the same area (Siddha et al. 2007).

This reported case was diagnosed as a sebaceous cyst after physical examination in another hospital before referral. After the excision biopsy, histopathological examination was done and showed focal malignancies features found in this case like areas with keratinization, enlarged cells with moderate to marked nuclear pleomorphism with vesicular nuclei, prominent nucleoli and abundant pale eosinophilic to clear cytoplasm. There were also many free *keratinous debris* noted. Numerous *foci* of calcification were identified within the tumor. Mitotic figures were frequently seen; they showed abnormal form. This leads to malignancy of proliferating pilar tumor that is very rare to be found.

In another case report based on Budrukkar et al. 2007, there was a histological examination result that was almost the same. There was a keratinized nodule. Nucleus showed hyperchromatic and pleomorphic with the present of multinucleated giant cells. There were mitotic figures that showed a malignancy of this tumor. There was also a distinct area, resembling a typical benign pilar tumor. It was composed of interlacing nodules of small peripheral cells that were matured into large central cells with central keratinization, so that it was diagnosed as a proliferating pilar tumor in the histological examination.

Sebaceous cyst grew from *infundibulum follicular* because of the plugging of keratin. Proliferating pilar tumor is a very rare tumor that appear from the external root of hair follicle. Both of them are derived from hair follicle with different etiologies, so that misdiagnosis might have happened, because from the appearance itself, this case looked like a *sebaceous cyst* without histopathological examination, because it is often diagnosed by just a physical examination.

This patient was referred from another hospital to Universiti Kebangsaan Malaysia Medical Centre because of malignancies found in histopathological examination. When operated in another hospital before coming to Universiti Kebangsaan Malaysia Medical Centre, there was no marginal resection or surgical margin, because it was just probably diagnosed as a benign *sebaceous cyst*. Surgical resection margin status is important to malignant lesions surgery.

Surgical resection margin is an area around the infected tissue that will take together with the tumor itself. This area can possibly have healthy cells or cancer cells, so that the surgeons must take the infected tumor cells together with the area around it called the surgical margin. In some hospitals, doctors want 2 millimeters (mm) or more of normal tissue around the cancer and the outer edge of the removed tissue, but it may vary in different hospitals to be a clear margin. Tumour free margins are the success key to the goal of treatment of tumor (Breastcancer.org, 2019).

A margin was called positive if an invasive tumor was cut by the surgical blade, but if the tumor was close and not transected, it can be considered as a negative for tumor by National Surgical Adjuvant Breast and Bowel Project (NSABP) B-06 study. A positive margin shows that there was a presence of tumor (Emmadi & Wiley 2012). In this case, because there was no margin resection in this patient, there was a re-excision of the residual tissue in the area that was previously operated in the previous hospital to prevent any residual cancer cells infecting the area.

After the patients underwent the surgery, there was a not-so-big wound after surgery, and there must be a dressing after surgery. The dressings should make a good environment for healing. The dressing should be painless and helps patients return to normal function (Imran et al. 2018). After that, a scar was formed in that area, because there was a wound healing after the surgery had been done. Wound healing is a natural process in human body to restore injured tissue. There are 4 phases of wound healing, namely hemostasis, inflammation, proliferation, and remodeling of the wound (Imran et al. 2011).

The patient's scar was a result of remodeling. Patients should take care of their wound after surgery even after the skin is healed, because these postoperative patients need to visit the doctors to focus on prevention and treatment of the scars. Before undergoing an operation, the patient needs to be aware of excessive scars formation. Sometimes, it can become a hypertrophic

scar and if the hypertrophic scar does not improve after six months, it will become a keloid. Keloid needs a variety of methods to treat. Keloid needs combination therapies to reduce it, because there are some of those that are effective to reduce it, and there are some are not effective. It is the same as for treating wounds, a combination treatment may be needed to heal the wound (Chik et al. 2016, Imran et al. 2011, Son & Harijan 2014).

This patient had a very rare malignant tumour, although the cyst had already been taken, but there will still be a re-excision for the residual tissue, because it turned out to be a malignancy and there was no margin resection. For malignancy, the patient must be thinking about it seriously and should be allowed to decide what happens to their bodies, so that the patient can recover soon (Muhammed et al. 2017). Pathologists in previous hospitals should need a certain diagnosis confirmation, because this case was a very rare case. The pathologists should coordinate with other hospitals to confirm this rare diagnosis by referring the patient to Universiti Kebangsaan Malaysia Medical Centre.

With this very rare case, it is hoped that pathologists can work together and communicate well to resolve this case. In addition, cooperation between surgeons and pathologists is needed for properly diagnosing this case, so that successful treatment can be achieved.

Strength and limitation

The case report provides a detailed description of a rare type of tumor, Proliferating Pilar Tumor (PPT), which can help increase awareness of this condition among healthcare professionals.The report highlights the importance of accurate diagnosis and histopathological examination in cases where a benign lesion such as a sebaceous cyst may be suspected but turns out to be malignant.The report can provide useful information for future case studies and research.The report does not provide information about the patient's medical history or potential risk factors for developing PPT, which may impact the prognosis and management of the condition.The report does not provide information about the patient's outcome or response to further management by a specialist team, which may be useful for future studies and patient care.

CONCLUSION

A 59 years old woman who was first diagnosed as epidermoid cyst after surgery and biopsy were done, the histopathological examination showed that there was proliferative pilar tumour with focal malignancy features. There will be future management by Universiti Kebangsaan Malaysia Medical Centre. This patient would have a re-excision of residual tissue in the area that was previously operated, because there was no surgical margin before. It will prevent patients from having residual cancer cells in that area.

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Conflict of interest

None.

Funding disclosure

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Author contribution

All authors contributed conseptual, study design, and analysis data. FHI was write the manuscript and final validation to publish.

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Review Article

THE ROLE OF DERMOSCOPY IN DIAGNOSIS OF BENIGN SKIN NEOPLASMS

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ABSTRACT

Benign skin neoplasms are commonly found in the population. It has a well-differentiated and slow growth nature. The patients often come seeking treatment when the tumor has developed into malignancy. This usual delay in diagnosis and therapy frequently happens because early-stage mass has not generated any complaints by the patients. Detection and monitoring of benign skin neoplasms can be carried out earlier and more effectively if the clinician or dermatologist has the knowledge of distinguishing benign from malignant lesions. The histopathological examination can help to establish the diagnosis, but this method is invasive and requires an extended amount of time. Dermoscopy is a practical, non-invasive and accurate method for early detection of skin disorder which reduces the number of unnecessary excisions of benign skin neoplasms. Knowledge of the vascular pattern and arrangement description, combined with the additional dermoscopic feature can lead to the prompt diagnosis of benign skin neoplasms.

Keyword: *Dermoscopy; benign skin neoplasms; diagnosis; diseases; tumor*

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Highlights:

1. Dermoscopy is a practical, non-invasive and accurate method for reducing the number of unnecessary excision of benign skin neoplasms.
2. Knowledge about vascular pattern and description setting can lead to a prompt benign skin neoplasm diagnosis.

INTRODUCTION

The incidence of skin neoplasms becomes more common, especially in the United States, Australia, and England. According to several studies, white people have a threefold increased risk of developing skin malignancies. In Indonesia, a tropical country where UV rays from the sun are quite intense and most people engage in activities that expose them to direct sunlight, the process of skin cancers is influenced (Rata 2016, Tsai & Dlugosz 2019).

Benign skin neoplasms are well-differentiated, grow slowly, and are frequently delayed in therapy. Benign skin neoplasms can be unsightly and maybe an indication of other disorder that can potentially be malignant. History taking, physical examination with the introduction of skin fluorescence, dermoscopy, and skin biopsy as the gold standard can all be utilized to assist in the identification of skin malignancies (Djuanda et al. 2016). Due to the lack of biopsy as a gold standard of diagnosis, understanding the practical features of many biopsy techniques, as well as the difficulties that can arise from a skin biopsy and how to handle them, is essential (Sonthalia et al. 2021).

The use of dermoscopy can improve the diagnostic accuracy of suspected skin malignancy in primary care physicians in several countries with sensitivity and specificity of 79.2% and 71.8% for dermoscopy, respectively, compared to 54.1% and 71.3% for naked eyes. The significant difference in sensitivity on dermoscopy was p=0.002 (Argenziano et al. 2006).

Dermoscopy is a link between microscopic and macroscopic aspects of a skin tumor that has a direct clinical and histopathological correlation. This reviewed the clinical aspects of common benign cutaneous neoplasms and recognized a classic hallmark in dermoscopy of some benign skin neoplasms. Furthermore, a review was necessary in order that doctors or dermatologists could recognize early management of skin therapy to prevent a malignancy or cancer of the skin.

OVERVIEW

Benign Skin Neoplasms

Tumor or neoplasms refers to swelling; an indication of inflammation, a pathological enlargement, or new growth of tissue with uncontrolled cell multiplication long after progressive stimulus has gone away. It is usually differentiated or mature with slow growth, no invasive or metastatic potential, does not spread to other parts of the body, and is usually isolated and encapsulated (Cuda et al. 2019).

Incidences and pathogenesis of benign skin neoplasms

The incidence of benign skin neoplasms varies in each population because the occurrence of neoplasms and their development is influenced by several factors, especially exposure to ultraviolet light and familial factors (Cuda et al. 2019).

A retrospective study showed that seborrheic keratoses (SK) ranked first for benign skin neoplasms (29.2%) out of 355 new patients with skin neoplasms (Hamzah & Effendi 2011). Another study on 482 patients (16.3%) with 21 types of benign skin neoplasms had found that among the group of patients with benign skin neoplasms, 132 patients (27.4%) verruca Vulgaris and 121 patients (25.1%) with SK were the most common benign skin neoplasms (Wijaya et al. 2011).

The etiology of skin neoplasms, whether benign or malignant, is not known with certainty, but there are factors that play an important role in the incidence of neoplasms in the skin, including internal and external factors. Internal factors include genetic, immunologic, race, and gender involvement. Irregularities in the expression of apoptotic markers p53 and Bcl-2 have been reported, although no genetic or chromosomal locus imbalance has been detected so far. Most individuals with skin neoplasms have a positive family history. External factors include carcinogens or chemicals (such as hydrocarbons, lead, nickel, etc.), sun exposure, environment, stress, and trauma/ infection (Tsai & Dlugosz 2019).

These internal and external factors predispose the local tissue to lose control over its growth at the gene level. Pluripotent epidermal cells and/or their epidermal or adnexal origin are assumed to be the source of the tumor (Djuanda et al. 2016).

Classification of benign skin neoplasms

Benign skin neoplasms are usually classed based on their origin, predisposition, clinical symptoms, and treatment options. Other research divides skin tumors into groups based on their histologic origin, age, location, and clinical characteristics. There is no uniformity in the classification system for benign skin neoplasms because of the varied origins and clinical features (Cuda et al. 2019).

The Indonesian Collegium of Dermatology and Venereology divides benign epidermal neoplasms, epidermal cysts, and adnexal benign neoplasms into benign epidermal neoplasms, epidermal cysts, and adnexal benign neoplasms; benign neoplasms of melanocytes and nevus cells; benign neoplasms of connective tissue; benign neoplasms of fat tissue and disorders of fat metabolism; benign neoplasms caused by viruses and vascular hyperplasia (Hamzah & Effendi 2011). The following types of benign skin neoplasms are frequently reported (Table 1).

Table 1. The most common type of benign neoplasms

Types of benign neoplasm	Tumor origin	Predilection site
Seborrheic Keratoses	Epidermis	Face Upper body
Nevus Pigmentosa	Neural crest	Face and body
Haemangioma	Blood vessel	Face, Trunk
Skin Tag	Fibrovascular tissue, epidermis, and dermis	Area of skin folds
Syringoma	Eccrine gland	Upper/lower eyelids Cheek Forehead
Xanthelasma	Lipid deposits	Eyelid
Keloids	Connective tissue Trauma	Deltoid area Chest Extremities
Solitary Trichoepithelioma	Hair follicle	Face, Body

Source: Djuanda et al. (2016)

The role of dermoscopy in benign skin neoplasms

Dermoscopy has been demonstrated to improve sensitivity for detecting skin malignancies when compared to naked-eye examination (NEE), with no loss of specificity. Essentially, dermoscopy allows biopsy specimens to be acquired from a smaller

number of lesions and this is reflected in the decreased number of benign lesions from which biopsy specimens are acquired for every skin cancer discovered (Yelamos et al. 2018).

Although skin biopsy is a simple outpatient procedure, complications such as bleeding, infection, and scarring may occasionally be encountered while performing a biopsy in an out-patient with basic infrastructure can occur and it is prudent to avoid them. When they occur, to recognize and manage them effectively (Killic et al. 2020, Sonthalia et al. 2021).

Minimizing factors such as improper lesion selection, poorly executed technique, unspecified clinical diagnosis, insufficient clinical information, faulty tissue fixation, and processing, improper staining for specific diagnoses, or insufficient collaboration between the dermatologist and the dermatopathologist. Furthermore, using dermoscopy to choose a biopsy site, as well as immunohistochemical staining and immunofluorescence procedures (if needed), can improve diagnostic accuracy (Korfitis et al. 2014, Ramsey & Rostami 2022).

Dermoscopy is an excellent tool for improving skin cancer diagnosis, as it offers high sensitivity for detecting skin malignancies while maintaining high specificity. A thorough understanding of dermoscopy is necessary; it can serve as a link between clinicians and pathologists, enhancing clinicopathologic correlation (Gulia et al. 2012, Yelamos et al. 2018).

Many diagnostic criteria and models have been established during the last decade to aid the proper detection of melanocytic lesions. Several studies have demonstrated that there are three algorithm approaches that can be employed and are accurate in distinguishing between benign and malignant skin lesions. The analysis pattern is based on extensive, qualitative evaluations of many factors on each individual. As a result, proper dermoscopy training is required (Gulia et al. 2012).

The physicians or doctors must be familiar with some dermoscopy terminology to do a dermoscopy examination. The terminology includes descriptive (clods, reticular lines, angular lines) and unique metaphors (globules, pigment network, rhomboid) (Kittler et al. 2016).

The following are various dermoscopy algorithms that can be used to distinguish between benign and malignant skin lesions (Table 2).

Table 2. Dermoscopic algorithms

Algorithm	Criteria	Description
ABCD rule of dermoscopy	Asymmetry, irregular borders, multiple colors, different dermoscopic structures within the lesion	A score >5.45 indicates a malignant lesion in a semiquantitative scoring method. A score of 5.45 indicates a benign lesion.
Seven-point checklist	Major criteria: network, blue-white veil, and atypical vascular pattern Minor criteria: irregular dots/globules, irregular streaks, irregular blotches, and regression structures	Each major criterion receives two points, while the minor criterion receives one point. Melanoma must be diagnosed with a total score of 3 or higher.
Three-point checklist	Asymmetry (streaks, dots/globules) Pigment network (typical, atypical) Blue-whitish veil	Critical evaluation of morphology and distribution of dermoscopic features

Source: Wang et al. (2012)

The role of dermoscopy in seborrheic keratoses

Seborrheic keratoses (SK) are keratinocyte neoplasms that are common and benign. They have distinct clinicopathologic characteristics. It most commonly affects people between the age of 40-50 years and over (Rata 2016). The hallmarks of clinical SK are flat lesions with obvious boundaries, light brownish to dark brown in hue, dull, and with pseudohorn cyst. All types of SK have a favorable family history. Seborrheic areas, such as the chest, back, abdomen, face, and neck, are frequently affected (Kittler et al. 2016).

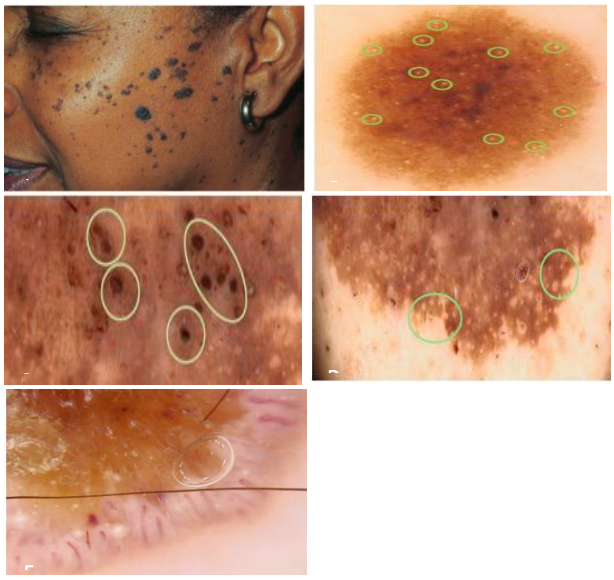

Figure 1. (a) clinical manifestations of SK, (b) dermoscopy appearance of the *milia-like cyst*, (c) *Comedo-like opening*, (d) *Moth-eaten border*, (e) *Hairpin-shaped*

After the advent of dermoscopy in 1998, the diagnostic accuracy for skin neoplasms and SK grew dramatically, attaining a sensitivity of 95.7% and a specificity of 78.3% (Carrera et al. 2017).

Dermoscopy, which looks for *milia-like cysts* (cloudy or starry), *comedone-like opening* (clods, brown, yellow, or orange lumps), *moth-eaten border*, fissures, and peaks, can assist to confirm the clinical diagnosis of SK. Another secondary dermoscopy feature of SK is the presence of well-defined blood vessels with a smooth superficial surface in a *hairpin-shaped structure* (Kittler et al. 2016). A study indicated that there were only 10% of the 203 SK were suspect and required histological confirmation (Carrera et al. 2017).

The role of dermoscopy in nevus pigmentosus

Nevus Pigmentosus is a benign skin tumor composed of nevus cells with skin disorders in the form of pigmentation. The location of these cells can be in the epidermis (junctional), the dermis (intradermal), or both areas (compound) (Rata 2016, Cuda et al. 2019). Nevus pigmentosus is a common problem since almost everyone has a nevus, with a 400% chance of turning cancerous if it changes. Nevus pigmentosus can affect any region of the body's skin, including mucous membranes close to the skin's surface.

Dermoscopy can reveal the deeper skin structure and characteristics of the junctional, compound, and dermal nevi, allowing for a better understanding of their prognosis. Dermoscopy has a sensitivity of 95.7% in nevus pigmentosus, making it a reliable alternative to the histological investigation. Dermoscopy features of nevus pigmentosus include typical pigment networks (lines, reticular, regular with minimal variations in color, thickness, and arrangement of pigment lines); *cobblestones* pattern*; starburst* pattern*;* and homogenous pattern (Wang et al. 2012, Kittler et al. 2016).

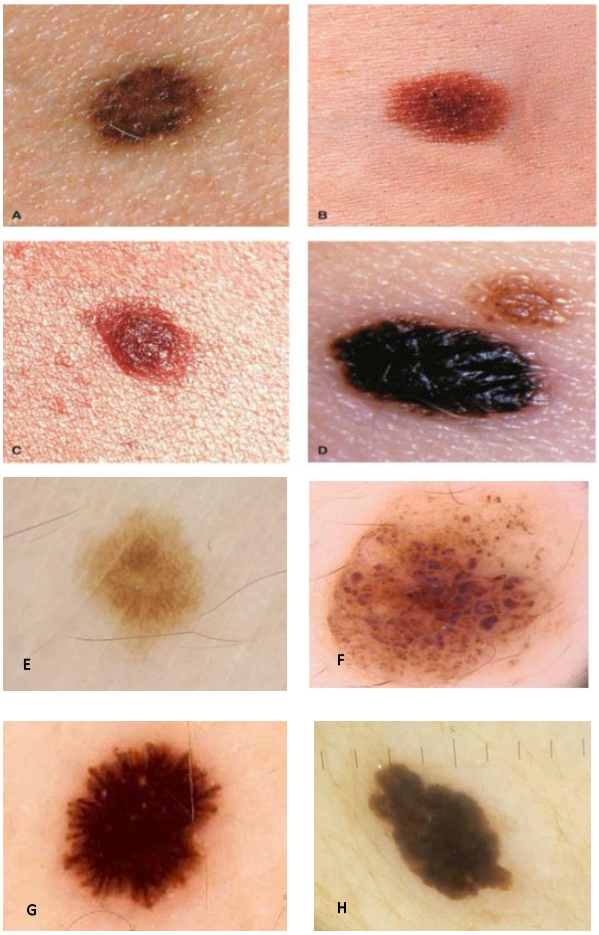

Figure 2. (a-d) clinical manifestations ofnevus pigmentosus, (e) dermoscopy appearance of typical pigment network, (f) cobblestones pattern, (g) starburst pattern, (h) homogenous pattern

The role of dermoscopy in hemangioma

Hemangioma is a benign tumor that develops in the skin, mucous membranes, and other organs as a result of abnormalities in the development and creation of blood vessels caused by endothelial cell proliferation. Hemangiomas are classified histopathologically as capillary hemangioma, cavernous hemangioma, or mixed capillary and cavernous hemangioma (Ahuja et al. 2013).

Hallmark dermoscopy hemangioma is the presence of *lacunae* characterized by well-defined, round, or oval areas, colored red, reddish-brown or reddish-blue, black separated from the stroma without other vascular

structures in it (red-purple *lacunes*) (Zaballoi et al. 2012).

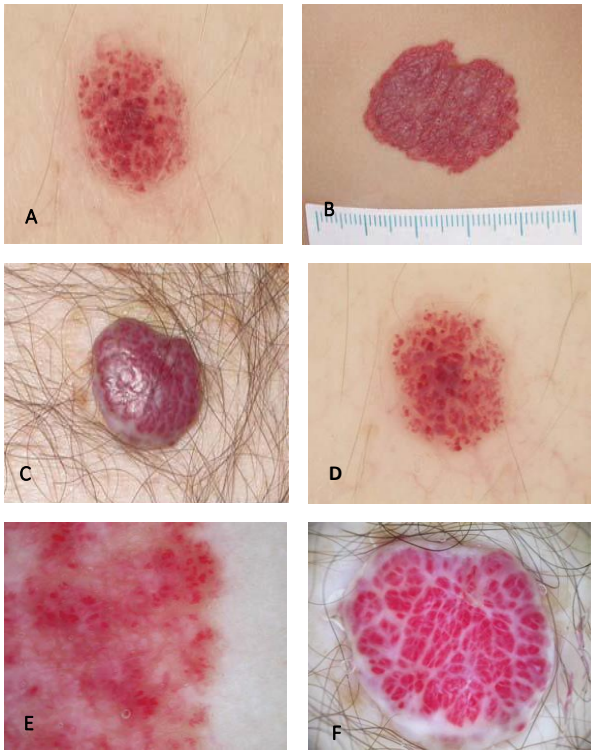

Figure 3. Clinical manifestations of *cherry* hemangioma (a) with appearance of *lacunae* in the middle and dilatation of peripheral blood vessels on dermoscopy (d); infantile hemangioma (b) with multiple, small, well-defined, homogenous red-white *lacunae* with red network of blood vessels on dermoscopy (e); other clinical manifestations of *cherr* hemangioma with multiple red *lacunae*, well-defined and tightly fused (f).

The role of dermoscopy in epidermal cyst

An epidermal cyst is one of the most common benign neoplasms, originating from the proliferation of epidermal cells and contains keratin. It is most common in areas rich in sebaceous glands, such as the face, neck, upper chest, and upper back. It is also linked to injuries to the palms and soles, as well as the buttocks. The lesion is a dome-shaped nodule with a central punctum that varies in diameter, is firm in substance, has a smooth surface, and is easily movable from the base yet adheres to the skin. The existence of a punctum, or pores of follicular origin, is a diagnostic clue, and epidermal cysts frequently mimic basal cell cancer (BCC) (Cuda et al. 2019).

The dermoscopy appearance of an epidermal cyst is a pore sign or comedo, and is a keratin-filled orifice that appears whitish, yellow, brown, or black in color (Figure 3c-d), and no characteristic features of arborizing BCC are found, such as vessels and large ovoid nests. Besides, a study reported in 83 patients with a diagnosis of epidermal cysts who underwent dermoscopy examination found 90% gave a pore sign (Ghigliotti et al. 2014).

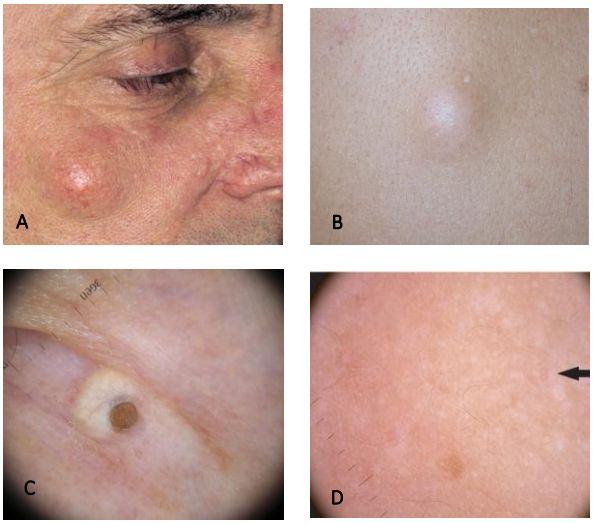

Figure 4. (a, b) clinical manifestations of epidermal cysts, (c) dermoscopy appearance of comedo with brown center and surrounded by yellow-white color, telangiectasia, (d) pore sign indicated by a black arrow

The role of dermoscopy in lymphangioma

Lymphangioma is a lymphatic vessel abnormality that often develops after birth. Lymphangiomas are divided into three types based on their clinical and histological characteristics there are localized circumscribed lymphangiomas, circumscribed lymphangiomas, and cavernous lymphangiomas. Circumscribed lymphangioma is the most frequent clinical type of lymphangioma, characterized by clusters of transparent, frog-like vesicles that develop shortly after birth or at any age. Lymphangia, hemangioma, angiokeratoma, and molluscum contagiosum are all possible diagnoses for this condition (Rata 2016, Zaballos et al. 2017).

Depending on the amount of blood in the vesicles, lymphangioma has a variety of dermoscopy patterns, characterized by a *lacunar* arrangement, clear fluid-filled lesions are light brown *lacunas* bordered by pale septa. When vesicles are blood-filled, dermoscopy characteristics vary depending on the amount of blood, localized crimson patches inside the lagoons for low blood content, pink diffuse coloration for a little amount of blood, or reddish to liliaceous *lacunar* structures for a larger amount of blood. In this situation, lymphangioma and hemangioma may be difficult to tell apart (Zaballos et al. 2017).

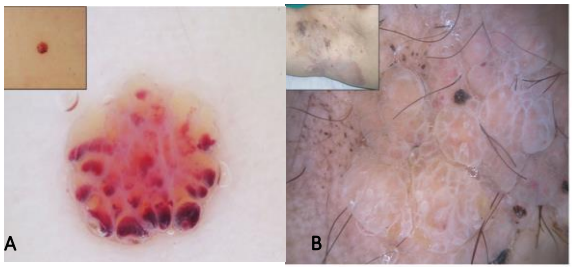

Figure 5. (a) clinical manifestations of circumscribed lymphangioma with *two-tone lacunae*, yellowish, with white lines and vascular structures, (b) yellowish *lacunae* with white stripes

The role of dermoscopy in angiokeratoma

Angiokeratoma is a benign hyperkeratotic vascular proliferation. Although the specific cause of angiokeratoma is unknown, causal factors include trauma, pregnancy, or tissue hypoxia. It clinically shows the presence of hyperkeratotic papules, solitary or multiple dark red to blue-black with a diameter of 2-10 mm, are present clinically. Five types of angiokeratomas are recognized angiokeratoma of Mibelli, angiokeratoma of Fordyce (angiokeratoma scrota), angiokeratoma corporis *diffusum*, angiokeratoma circumscriptum *naeviforme*, and solitary angiokeratoma (Sadana et al. 2014).

Dark *lacune*, whitish veil, erythema, peripheral erythema, red *lacunae*, and hemorrhagic crusts were all seen in at least 50% of single angiokeratomas. The most accurate pattern for a correct diagnosis of angiokeratoma is dark l*acunae* with sensitivity and specificity of 93.8% and 99.1%, respectively (Papageorgiou et al. 2018).

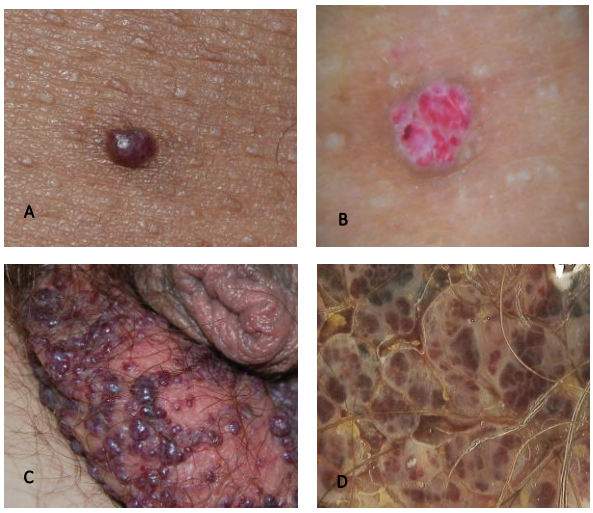

Figure 6. (a) clinical manifestations of solitary angiokeratoma, (b) dermoscopy appearance of red *lacunae*, (c) scrotal angiokeratoma (c), (d) with a whitish veil

Strength and limitation

The topic of early detection and monitoring of benign skin neoplasms is important for both patients and healthcare providers.The article acknowledges the delay in diagnosis and therapy that often occurs with these types of tumors, highlighting the need for improved detection methods.The article does not provide specific data or research to support its claims about the effectiveness of dermoscopy in detecting and monitoring benign skin neoplasms.

CONCLUSIONS

Recognition and finding of benign skin neoplasms lesions are highly essential to assist in establishing the diagnosis and treatment. Dermoscopy is one of the diagnostic tools used to aid in the diagnosis of benign neoplasms. It was useful in clarifying the patterns and structures in the lesion, resulting in a more accurate clinical diagnosis, and allowing for more appropriate therapy in determining the extent of the lesion that requires excision and some benign neoplasms have a classic hallmark that could improve diagnostic accuracy.

Conflict of interest

None.

Funding disclosure

None.

Author contribution

IP contributed conseptual, collecting data, and analysis data. MS was write the manuscript. IP was final checking content.

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Review Article

THE AKT PATHWAY AND SATELLITE CELL ACTIVATION IN SKELETAL MUSCLE MASS REGULATION

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ABSTRACT

Muscles have an important role as a regulator of glucose and triglyceride metabolism. Some researches show the correlation between skeletal muscle mass and metabolic diseases, such as diabetes. Skeletal muscle mass decrease occurs due to chronic illness or physiological process of aging, thus increasing the risk of metabolic diseases as well as motion difficulty in the elderly. Skeletal muscle mass depends on balanced protein synthesis and degradation, controlled through a variety of signal transduction pathways including the AKT. AKT or protein kinase B increases protein synthesis through the mTOR and GSK3β and controls the degradation of proteins through FoxO transcription factors. Another factor that has an alleged role in the regulation of skeletal muscle is the satellite cells which provide remarkable ability to regenerate skeletal muscle. A comprehensive understanding of the biomolecular mechanism of muscle mass regulation is important to develop effective treatment or prevention of muscle atrophy in many cases, either caused by pathological conditions, such as chronic diseases, or the process of aging.

***Keywords**: Skeletal muscle mass; AKT pathway; satellite cells; human & health*

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Highlights:

1. AKT or protein kinase B increase protein synthesis and control the degradation of proteins.
2. Satellite cells was provided remarkable ability to regenerate skeletal muscle.
3. Stimulation of protein synthesis is effective therapy to maintain muscle mass, prevent muscle wasting to reduce risk sarcopenia and improve quality of life in the elderly.

INTRODUCTION

Skeletal muscle is a major part of the human body. Skeletal muscle has several important functions, such as maintaining posture, movement and regulating glucose and triglycerides metabolism. Skeletal muscle is the main organ for glucose deposition. Skeletal muscles convert glucose into energy to generate movement and activity. Decreasing of skeletal muscle mass has been connected to chronic diseases, such as diabetes mellitus (Collins et al. 2018, Hong et al. 2017). Insulin resistance in muscle is the main characteristic of metabolic diseases, such as obesity, type 2 diabetes (DM 2), and metabolic syndrome. Skeletal muscle mass was thought to have a protective role against metabolic diseases (Lee et al. 2019).

The decrease of skeletal muscle mass also occurs physiologically in the aging process. Skeletal muscle mass decreases approximately 8% per decade up to 70 years and decreases 15% per decade after 70 years. Consequently, it leads to various problems in the elderly, such as immobility, fragility as well as increasing the risk of metabolic diseases (Gomes et al. 2017).

Skeletal muscle mass controlled by various molecular signaling pathways. Those signals regulate the balance of muscle protein synthesis and degradation. Altered muscle regulation resulted in muscle hypertrophy and atrophy. Signaling pathways regulate the growth, regeneration and regulation process of muscle mass since the embryonic period (Egerman & Glass 2014). Comprehensive understanding about the underlying molecular mechanism that regulates skeletal muscle mass is important as the basis for therapeutics research about sarcopenia in the aging population. The purpose of the therapeutic agent is to increase the mass and prevent muscle atrophy, thereby lessening the risk of various chronic diseases, reducing the burden of care and improving quality of life.

OVERVIEW

Skeletal muscle regulation and protein synthesis

Skeletal muscle has a specific structure for its typical function. Skeletal muscle fiber (myofibrils) are composed of proteins including actin, myosin, titin and other proteins that hold them together. These proteins are organized into myofilaments. A bundle of myofibril covered by epimysium forms a muscle fiber and bundles of muscle fibers constitute skeletal muscle tissue. About 80% of the muscle fiber is composed of proteins (Frontera & Ochala 2015). Muscle mass balance mechanisms are influenced by various factors, such as nutritional status, hormonal, physical activity, and injury or diseases (Cai et al. 2016).

Skeletal muscle hypertrophy is characterized by myofiber enlargement with no change in myofiber number or hyperplasia. Significant changes in skeletal muscle hypertrophy are the increases of protein synthesis, while muscle mass loss occurs through the protein degradation. Genetics regulation determines the physiology changes in muscle activity. Various signal transduction pathways influence protein synthesis and degradation, thus can control muscle growth (Moriya & Miyazaki 2018). An anabolic stimulus, such as Insulin Growth Factor (IGF) can trigger muscle hypertrophy, while myostatin and other growth factors, such as members of transforming growth factor (TGF-β) have an inhibitory effect on muscle growth (Egerman & Glass 2014).

Skeletal muscle increased through the AKT pathway

The muscle protein synthesis and degradation processes were controlled by several pathways of molecular signals, such as *phosphatidylinositol* 3-kinase (PI3K)/ AKT (serine/*threonine-kinase*) pathway. Activation of AKT, also known as PKB (Protein Kinase B), mediated a variety of cellular functions including angiogenesis, metabolism, growth, proliferation, cells survival, protein synthesis, transcription, and apoptosis (Hemmings & Restuccia 2012).

PI3K/ AKT pathway mainly roled protein synthesis and inhibited protein degradation, so that AKT/ PKB was an important pathway in muscle hypertrophy. PI3K/ AKT pathway had been proven to be the main pathway of muscle hypertrophy. Studies using genetic approaches had shown expression of either PI3K or AKT induced muscle fiber hypertrophy both in vivo and in vitro (Cai et al. 2016, Hemmings & Restuccia 2012).

The AKT pathway worked through various channels by activation or inhibition of its effectors that could ultimately maintain the protein synthesis. AKT inhibited protein degradation by suppressing the transcription factor forkhead box O (FoxO) and stimulated protein synthesis through mechanistic target of rapamycin (mTOR) and glucose synthase kinase (GSK3β). AKT could be activated by phosphorylation after a series of an intracellular signaling cascade that involved growth factors, such as IGF-1 and PI3K. PI3K is a lipid kinase that regulates the levels of phosphorylated *phosphatidylinositol* (PIP3) at plasma membrane. PIP3 acted as docking sites for the two kinases, such as phosphoinositide-dependent kinase 1 (PDK1) and AKT (Egerman & Glass 2014, Hemmings & Restuccia 2012).

PI3K activated AKT and mTOR, which regulated the metabolic activity of cellular biosynthesis supporters. mTOR regulates protein translation control through emultiple effectors. AKT indirectly affected mTOR by inhibiting protein tuberous sclerosis complex (TSC) 1 and 2 as a GTPase activating protein (GAP) that inactivated small G protein Ras (Rheb) which could activate the mTOR directly (Yoon 2017).

mTOR has two different complex biochemical structures that are bound to the raptor mTORC1 and mTORC2 bound by Rictor. mTORC1 is highly sensitive to rapamycin and regulates protein metabolism and autophagy. mTOR stimulation can initiate the process of protein translation and can be stimulated by some nutritional compounds, such as amino acids, insulin, growth factor and muscle contraction (Santos et al. 2017, Yoon 2017).

PI3K/ AKT activation mechanism

Muscle hypertrophy mechanism through activation of P13K/ AKT by IGF1 and insulin receptor IGF1 starts with ligand bound to its receptor located on the cell membrane. IGF 1 receptor is a tyrosine kinase receptor called as IGF1 (IGF1- R). This bonding caused phosphorylation of tyrosine kinase receptors which then formed a docking site to the insulin receptor substrate (IRS-1). Muscle hypertrophy signaling pathways through IRS- IGF1 was an important mediator for IGF signaling pathway (Andrade et al. 2017). Phosphorylated IRS acts as the mounting location to move and activate PI3K, which phosphorylated membrane phospholipids and generated phosphoinositide-3,4,5-triphosphate (PIP3) from phosphoinositide-4,5-bisphosphate (PIP2). The next cascade of autophosphorylation activated the AKT (Hemmings & Restuccia 2012).

In addition to initiating the synthesis of protein, AKT inhibited protein degradation by suppressed family transcription factor FoxO1, FoxO3, and muscle ring finger 1 (MuRF1). FoxO was an inhibitor of cell survival factor that stimulated the synthesis of proteins through mTOR and glycogen synthase kinase 3β (GSK3 β) (Egerman & Glass 2014). GSK3 β phosphorylation causes the release of a ribosomal translation inhibitor factor called eukaryotic translation initiation factor 2B (EIF2B) (Yoon 2017). Activation of mTOR could stimulate the activation of p70S6K that led to the ribosome s6 phosphorylation, so that the protein translation process could occur.

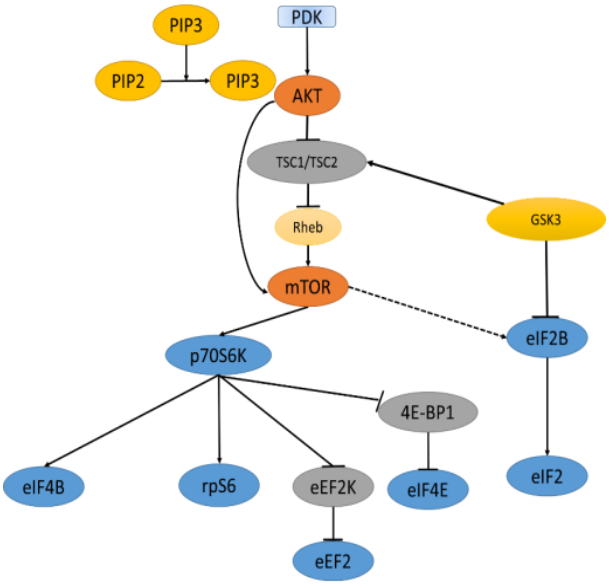

Figure 1. AKT pathway/ TSC2/ mTOR in protein synthesis (Favier et al. 2008)

Note: Activation of phosphatidylinositol-3-kinase (PI3K) led to phosphorylation of phosphatidylinositol-biphosphate (PIP2) into phosphatidylinositol-triphosphate (PIP3) as the docking site membrane for two kinases: serine/ threonine kinase AKT (or protein kinase B) and protein kinase (PDK). AKT phosphorylated by PDK and thus enabled the translocation to the membrane. Once activated, AKT phosphorylates tuberous sclerosis complex (TSC) 2, which joined the TSC1 and caused the release of inhibition of the Ras homolog (Rheb).

Rheb directly activates mTOR which then stimulates ribosome biogenesis, mRNA initiation and elongation via the phosphorylation of the 70-kDa ribosomal protein, S6 kinase (p70S6K) and inhibit 4E-BP1 binding to eukaryotic initiation factor (eIF) 4E. In addition, Akt promotes protein translation via inhibition of glycogen synthase kinase (GSK) 3, which controls the activity of eIF2B. TSC2 enabled by GSK3 and inhibited by Akt.

Stimulation of serine 6 kinase 1 (S6K1) activity by mTORC1 could induce mRNA biogenesis processes, including transcription, elongation and protein translation on the ribosome. The ribosomal protein translation process is activated by the eukaryotic initiation factors (eIF4E), which in normal conditions bind to the binding protein 4E-BP1. Phosphorylation of 4E-BP1 by mTOR reduced the affinity of 4E-BP1 to eIF4E. The release of eIF4E from 4E-BP1 could initiate the translation process (Hemmings & Restuccia 2012). Therefore, the AKT-GSK-3β and AKT-mTOR pathway is important to increase protein synthesis associated with muscle hypertrophy.

Satellite cells functioned on muscle regeneration

Skeletal muscle has a great ability to adapt to the functional demands and regenerate after injury. This remarkable ability was regulated by satellite cell. Satellite cells are spindle-shaped mononucleus cells located under the basal lamina between fiber sarcolemma, and surrounded by mature muscle fibers. Satellite cells can only be recognized with an electron microscope and regarded as inactive sedentary myoblasts after the muscle differentiates. It mainly functioned to renew muscle fibers after trauma or in life after birth (Almeida et al. 2016).

Maintenance of muscle mass depends on the balance between protein synthesis and degradation. However, some studies revealed that satellite cells took a role in cell turnover, muscle growth and maintenance of muscle mass. Satellite cells played a role as the main donors of new nuclei, into myogenic precursor cells that are important for muscle growth and regeneration. Satellite cells contributed in the process of muscle hypertrophy in response to exercise and hormonal stimulation (Musarò 2014).

The involvement of satellite cells in muscle hypertrophy in adulthood is still debated, although theoretically the addition of new myonuclei caused by satellite cells and satellite cell fusion could occur during myofiber hypertrophy. A study conducted on

mouse models with AKT transgene showed muscle hypertrophy that was not accompanied by satellite cell proliferation (Blaauw et al. 2009). Physiologically, the satellite and myonuclei cells can undergo apoptosis during muscle atrophy, although myonuclear loss occurs in muscle atrophy is unclear. Increasing the size of myofiber can also occur in the cells regeneration process, but not in the postnatal hypertrophy phase. However, a different research showed that the body mechanism to maintain muscle mass needed the presence of the satellite cell in quiescent condition. A study using diphtheria toxins to diminished satellite cells showed a decrease in skeletal muscle mass (Sambasivan et al. 2011). Another study showed satellite cell proliferation and differentiation followed muscle hypertrophy induced by endurance and resistance exercise (Bazgir et al. 2017).

Satellite cell activation mechanism

In the off-state, satellite cells were in the G0 phase. In this condition, the satellite cells expressed myogenic factor 5 (Myf5). When an injury or other stimuli occured, the silent satellite cells were activated, then proliferated and combined to form a new skeletal muscle fiber. The satellite cell activation was done through the transcription factor, paired box 7 (Pax7) (Musarò, 2014). The proliferation and myoblast Proliferative activity of IL-6 contributed to the growth of hypertrophic myofibers. IGF-1R signaling in cell regeneration and activation of satellite cells was highly important. A study conducted in vivo with heterozygote mice IGF-1R (IGF-1R +/-), showed a decrease in expression of MyoD and myogenin as a marker of proliferation and differentiation of satellite cells (Dong et al. 2013). IGR-IR activated satellite cells indirectly through the AKT pathway which had an important role in the myoblast differentiation process.

daughter fusion is marked by myogenic differentiation (MyoD) expression as a marker of active satellite cell proliferation. A small part of the satellite cells was then deactivated by downregulation of MyoD and was used as an independent regeneration of satellite cells, MyoD expression was induced in 24 hours on the satellite cell activation (Endo 2015, Musarò 2014).

Afterwards, satellite cells that have migrated to the injured site proliferated to produce a number of myoblasts needed for regeneration. Some growth factors and cytokines played an important role in this process, including HGF, FGF, IGF-1, LIF, and IL-6 released from injured muscle or infiltrated phagocytes (Musarò 2014). IGF-1 was produced by satellite cells, myofibers and liver and acted in autocrine, paracrine and endocrine systems. IGF-1 did pleiotropic functions, such as in the satellite cells or myoblasts proliferation, differentiation, maturation and hypertrophy during muscle regeneration. All these functions were mediated by IGF-1 receptor (IGF1R). IGF1 signaling - IGF1R activated the Ras-ERK pathway and signaling (Ras-) PI3K-AKT, so that it could process proliferation and differentiation. IL-6 secreted from myofibers also stimulated proliferation of satellite cells through activation of STAT3.

Some studies conducted in animal models of muscle atrophy showed satellite cell activation by an increase in the expression of MyoD through the AKT pathway (Hauersley et al. 2014). The similar result found in vitro experiment with black ginseng also showed an increase in the expression of MyoD and MHC through the AKT pathway (Lee et al. 2018). Another animal research using AKT1 KO mice treated by mechanical load indicated that the AKT pathway was important to induce satellite cell proliferation (Moriya & Miyazaki 2018).

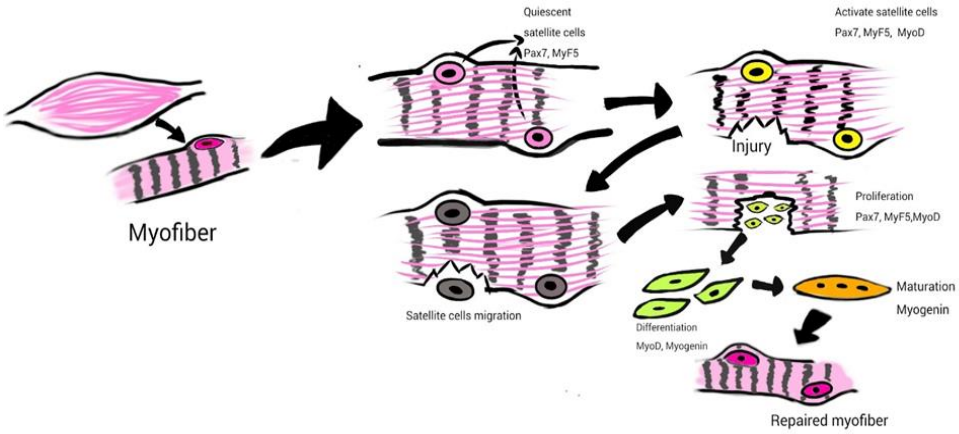

Figure 2. Skeletal muscle regeneration process
(Endo 2015)

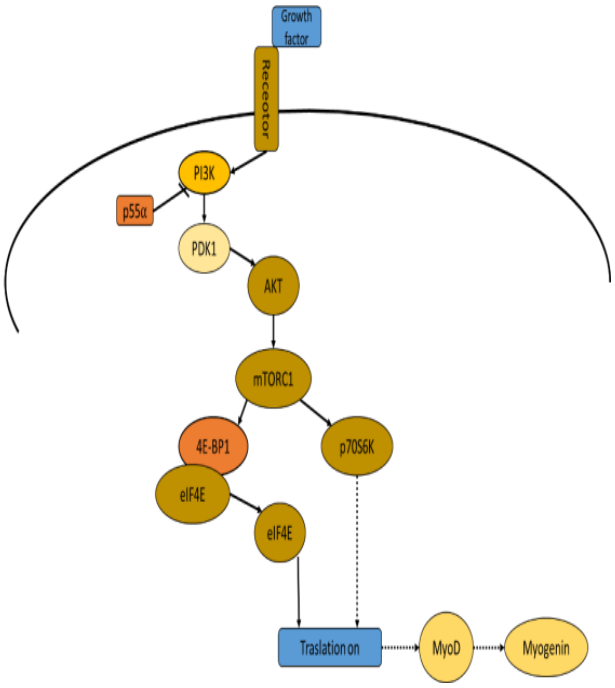

Figure 3. AKT signaling pathway in the activation of satellite cells (Hauersley et al. 2014)

Strength and limitation

The role of AKT and satellite cells in regulating skeletal muscle mass is explained, providing insight into the molecular mechanisms behind muscle atrophy and potential targets for treatment or prevention.The importance of understanding muscle mass regulation in both pathological conditions and aging is acknowledged, indicating the relevance of this topic for a wide range of individuals. The statement does not provide any specific research examples to support the correlation between skeletal muscle mass and metabolic diseases, making it difficult to assess the strength of the evidence presented.

CONCLUSION

AKT pathways regulated muscle mass by increasing protein synthesis through mTOR and GSK3β. Other than the protein synthesis regulation, the AKT was also important to the activation of the satellite cells and suggested involvsement in skeletal muscle hypertrophy. AKT pathway activation was done through a variety of growth factors that could be a ligand to activate the insulin receptor (IRS). The AKT pathway activation was mainly carried out through physical activity, especially resistance training or lifting weights. In addition, the intake of certain supplements had a potency to activate the AKT pathway, thus allegedly able to provide the same effects as physical activity to increase muscle mass through the protein synthesis stimulation. This could be an effective therapeutic option for maintaining muscle mass and preventing muscle wasting, so that it can reduce the risk of sarcopenia and increase the quality of life in the elderly.

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Conflict of interest

None.

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Author contribution

SS-conseptual and study design, VMT-collected data, RL-analysis data. HG was write the manuscript and final check.

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Review Article

THE EFFECT OF ORLISTAT ADMINISTRATION IN CHANGE OF GLYCEMIC CONTROL AND WEIGHT LOSS OF OBESITY OR OVERWEIGHT PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Risk of Type 2 diabetes mellitus (T2DM) increases steadily with increasing overweight and obesity, and these two-health problems are emerging epidemics worldwide. Orlistat, a lipase inhibitor for weight loss drug, is often used in T2DM medication as adjuvant therapy, but effectiveness of the drug for improving glycemic control on T2DM patients is unclear. This study was to determine the effect of orlistat on glycemic control and weight loss in overweight or obese patients with T2DM. Term "Orlistat" AND "(obesity OR overweight)" AND "(HbA1c OR A1C)" AND "diabetes" were systematically searched in Pubmed and Science Direct web databases up to March 2021. Only randomized controlled study (RCT) methods studies were included in this study. Collected final samples were presented in a table with narrative review. There were 9 RCT studies with a total 2,175 subjects that met inclusion criteria. Of the sample, 360 mg/day orlistat as an adjuvant therapy, was administered to overweight or obese T2DM patients together with hypocaloric intake (8 studies) or without hypocaloric intake (1 study) intervention. They were examined for 12-52 weeks. From 2 short-term (12 weeks) studies, one study revealed that orlistat improved HbA1c and fasting plasma glucose (FPG) level significantly, while one study showed no significant effect compared to placebo. Seven other studies (long term observation) had found that orlistat significantly improved HbA1c and FPG level. All studies found that orlistat significantly reduced body weight. As an adjuvant therapy, Orlistat improved HbA1c and FPG level in overweight and obese T2DM patients.

***Keywords**: Orlistat; type 2 diabetes mellitus; overweight; obesity; glycemic control; body weight*

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Highlights:

1. The effect of orlistat on glycemic control and weight loss in overweight or obese type 2 diabetes mellitus patients was determined.
2. HbA1c and FPG level in overweight and obese type 2 diabetes mellitus patient can improved by orlistat as an adjuvant therapy.

INTRODUCTION

The incidence and severity of metabolic disorders of type 2 diabetes mellitus are closely related to obesity and overweight (Wannamethee & Shaper 1999). The implication of the continuing increase in the prevalence of obesity and overweight for the incidence of type 2 diabetes is a serious condition (Kumanyika et al. 2002). The incidence of diabetes increased in obese people (18.0% to 20.1%), indicating that most of the increase in diabetes prevalence was due to the increase in obesity prevalence. In fact, 85.2% of people with type 2 diabetes mellitus are overweight or obese (Bhupathiraju & Hu 2016). One logical approach, but difficult to achieve in practice, to prevent or treat type 2 diabetes is the long-term management of overweight. Several studies have shown that moderate weight loss of 5–10% through diet and lifestyle interventions

substantially increase the risk profile for cardiovascular and glycemia and improves the risk of type 2 diabetes mellitus in high-risk subjects (Williamson et al. 2000).

Orlistat is a lipase inhibitor, originally developed for long-term management of obesity which selectively inhibits digestion and absorption of triglycerides in the digestive tract. At a dosage of 120 mg three times daily combined with a mildly hypocaloric diet, orlistat reduces dietary fat absorption by about 30% (Hanefield & Sachse 2002). The therapeutic application of orlistat is not just a weight-loss treatment. In combination with lifestyle intervention, orlistat reduces the development of impaired glucose tolerance and progresses to type 2 diabetes in obese patients. Besides, weight loss induced by orlistat in association with a low-calorie diet is accompanied by improved glycemic control and cardiovascular risk factors in obese patients receiving treatment for type 2 diabetes (Hanefield & Sachse 2002).

Until now, the use of orlistat as adjuvant therapy in obese patients with type 2 diabetes mellitus is still controversial. Some studies proved that giving orlistat could significantly reduce body weight, HbA1c, and GDP, but there were still studies which indicated that weight loss and HbA1c as the result of giving orlistat was less significant compared to placebo (Derosa et al. 2011, Hanefield & Sachse 2002).

Another systematic review study conducted by Aldekhail et al. (2015) showed that orlistat had a significant effect on body weight, HbA1c, and GDP in diabetic patients in the short-term (<3 months). However, this systematic review was unable to demonstrate the long-term effectiveness of orlistat (Aldekhail et al. 2015).

This study aimed to review the effect of orlistat in obese or overweight patients with diabetes in the short-term and longer-term based on several previous studies by comparing several existing studies.

MATERIALS AND METHODS

This study used cross sectional study design with a systematic review method based on several randomized control trial studies. The inclusion criteria in this study were obesity (BMI >25 kg/m^2), aged >18 years, diagnosed with type 2 diabetes mellitus, and was currently on diabetes treatment or had just been given diabetes treatment at the beginning of the study. The exclusion criteria of this study were discontinuation of T2DM treatment when the study was started.

Data collection techniques in this study came from nine scientific papers published in English language international journals. The search was carried out using several search engines, such as Pubmed and ScienceDirect which focused on some keywords, namely Orlistat AND (obesity OR overweight), AND (HbA1c OR A1C), and AND diabetes conducted until March 29, 2021.

The sample used in this study were 9 RCT with a total of 2,175 research subjects from Pubmed and Science Direct regarding the effect of orlistat on changes in glycemic control and weight loss for obese or overweight patients with type 2 diabetes mellitus. The collected data were managed using PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) method and using Zotero software to manage reference sources.

RESULTS

The search identified 206 potentially eligible citations, of which one was excluded as duplicates (Figure 1). On reviewing the titles and the abstract of 205 articles, 176 were considered English language articles and the full articles were obtained. The 176 articles, and 12 fulfilled the inclusion criteria. Two studies were excluded for discarding diabetes treatment during the study (Kelley et al. 2003, Kopelman et al. 2010). One study was excluded for using both diabetic and non-diabetic patients as subjects (Hanefield & Sachse 2002). The final 9 selected RCTs were published between 1998 and 2012 (Table 1).

The number of participants in each study ranged from 60 to 503. The pooled group comprised 2,175 participants, including 1,083 participants in the orlistat treatment group and 1,092 participants in the control group. Two of the studies were conducted in China (22%) (Kuo et al. 2006, Shi et al. 2005), and one each in the other eleven countries. Studies varied in duration between 12 and 52 weeks. Studies were divided by the duration of the study. Two studies were conducted for ≤12 weeks categorized into short-term studies (Derosa et al. 2011, Kuo et al. 2006), and other studies categorized into long-term studies. All of the studies used orlistat at a dose of 360 mg/day. One study used moderate diet (Kuo et al. 2006), and the other studies used hypocaloric diet. No specific physical activity information was provided in all studies.

The mean BMI values at baseline of the included studies for orlistat and control group ranged from 26.9 (study conducted in China) to 35.2 and 27.2 (study conducted in China) to 35.6. One study did not provide BMI values at baseline, but the subjects BMI values ranged from 25 to 40 (study conducted in China) (Shi et al. 2005). All studies reported results for weight (kg), HbA1c and FPG, each baseline and changes (Table 2).

The overall mean HbA1c and FPG levels decreased more in the treatment groups than in the control group. In short-term studies, a greater mean HbA1c and FPG change was reported in lifestyle intervention with orlistat treatments compared to lifestyle intervention with placebo ($p<0.05$), although one study showed no significant changes in HbA1c and FPG (Derosa et al. 2011). In long-term studies, the reduction in HbA1c and FPG in orlistat treatment groups was greater than the reduction in control groups ($p<0.05$). Short-term studies showed a greater mean HbA1c change than long-term studies. The overall mean weight loss was greater in the treatment groups than in the control group ($p<0.05$), either in short-term group or in long-term group.

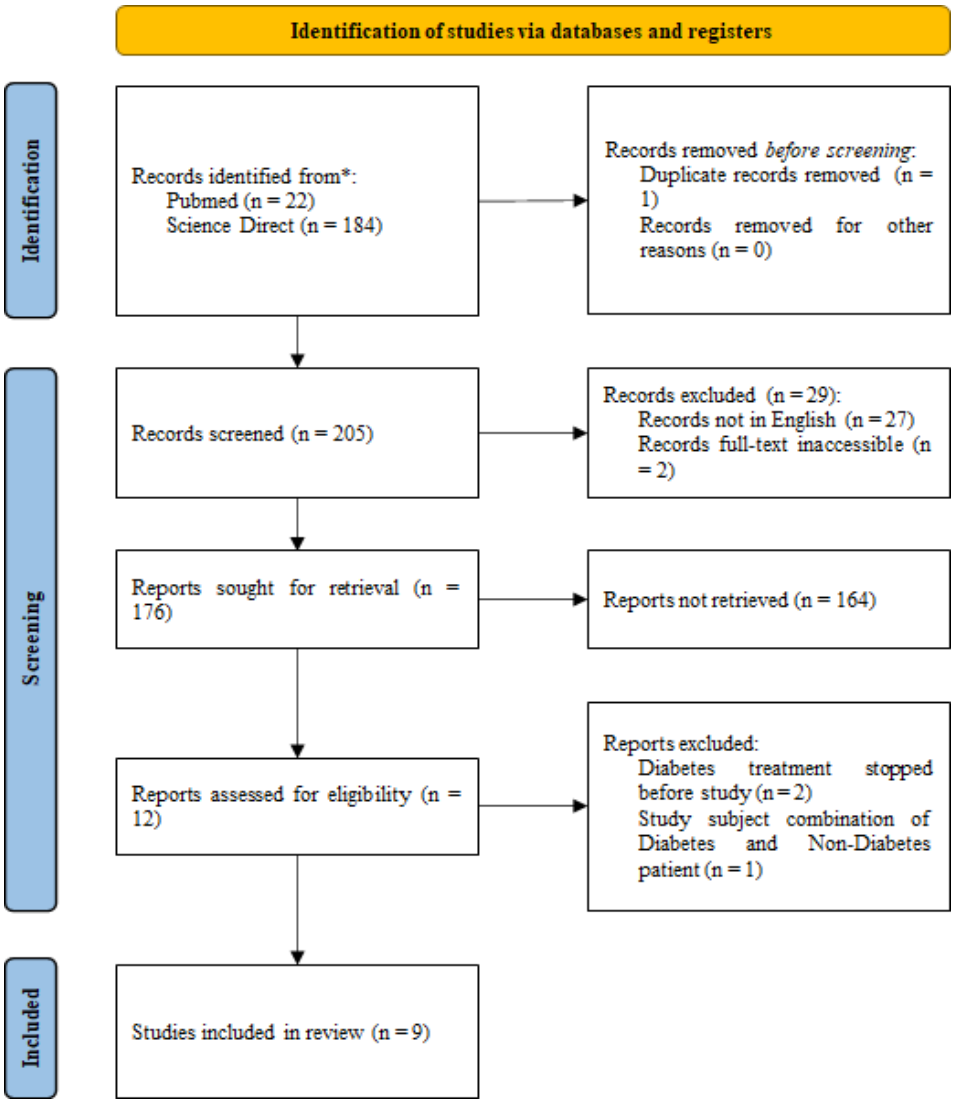

Figure 1. The PRISMA 2020 flow diagram

Table 1. Characteristics of studies included in systematic review

Authors/Year of Publication	Country	Population (n)		Age	BMI (kg/m^2)	Follow-up
		Orlistat	Placebo			
Hollander/1998	United States	162	159	> 18	28 – 40	52 weeks
Miles/2002	United States, Canada	249	254	40 – 65	28 – 43	52 weeks
Berne/2005	Sweden	111	109	30 – 75	28 – 40	52 weeks
Lindgarde/2000	Germany	68	60	18 – 75	28 – 38	48 weeks
Halpern/2003	Brazil, Argentina, Colombia, Costa Rica, Mexico	139	141	18 – 70	≥ 27	24 weeks
Guy-Grand/2004	France	97	96	18 – 65	≥ 28	24 weeks
Shi/2005	China	117	119	18 – 65	25 – 40	24 weeks
Kuo/2006	China	30	30	≥ 18	≥ 24	12 weeks
Derosa/2011	Italy	113	121	≥ 18	≥ 30	12 weeks

DISCUSSION

Given that the prevalence of obesity and diabetes continues to increase, more efficient treatment is needed, such as using an alternative orlistat for patients with type 2 diabetes mellitus with obesity. In general, administration of orlistat did not only reduce weight, but also improved glycemic control. Orlistat which worked to reduce fat absorption in the gastrointestinal system by inhibiting lipase had been shown to reduce body weight.

Obesity is closely related to insulin resistance. This was consistent with the mechanisms of orlistat through increased insulin sensitivity, slower and incomplete digestion of dietary fats, reduction of postprandial plasma non-esterified fatty acids, decreased visceral adipose tissue, and stimulation of peptide-1 secretion, such as glucagon in the lower small intestine (Aldekhail et al. 2015). This was proven by this review which showed the same result. Moreover, orlistat caused significant reduction in weight, BMI, waist circumference in obese adults compared to placebo (Jain et al. 2011). Orlistat also increases the proportion of people who lose 5% or more of their body weight, while on an energy deficit diet (Chauhan et al. 2011).

Our systematic review used 9 valid RCT studies. Of these studies, orlistat showed that it could decrease body weight, HbA1C, and FPG level in both short- and long-term administration. However, there were variations in outcomes between short- and long-term studies compared to placebo. This might be due to interventions not only in the form of offering orlistat, but also changing lifestyle, such as low-calorie diet and 30 minutes of exercise for 5 times a week or cycling. Low-calorie diet and exercise can increase weight loss, so that it has an impact on increasing insulin sensitivity as measured by controlled HbA1C and FPG levels.

The success of orlistat in significantly losing weight compared to placebo was found in 9 studies (100%) which included two short-term studies (100%) and seven long-term studies (100%). Meanwhile, the impact of orlistat treatment on HbA1c and FPG compared to placebo was found to be significant in one short-term study (50%) and seven long-term studies (100%), so that it seems that orlistat effected more clearly to improve glycemic control when administered in long-term (>12 weeks). This was in accordance with another study that orlistat significantly decreased FPG and HbA1C compared with placebo-treated patients (Jacob et al. 2009). In fact, orlistat still provided significantly greater decrease for both and HbA1c for patients with minimal weight loss (1% of baseline body weight). According to other research, orlistat appeared to reduce the need for concomitant diabetes medication irrespective of weight loss (Rowe et al. 2005).

One study showed different results where there was no significance in the decrease of HbA1c and FPG compared to placebo (Derosa et al. 2011). This might be because the study population were those whose blood sugar was difficult to control even with insulin administration, because they had received antidiabetic drugs or insulin, but their blood sugar remained uncontrolled.

The comparative study between short-term and long-term administration showed that the weight loss and FPG reduction varied between two groups. However, the HbA1c reduction showed that the short-term administration provided greater results than long-term. This might be due to decreased lifestyle adherence that decreased over time and deteriorating glycemic control that occurred in long-term administration of orlistat, such as the decrease was not as optimal as during the first three months. This result was comparable with a previous study of the effect of orlistat in glycemic control conducted by Aldekhail et al. (2015) which showed the largest decrease in HbA1c levels in the first three months and was followed by a mild increase thereafter, although it was still followed by weight loss for up to 12 months.

Table 2. Effect of orlistat administration toward HbA1c, FBG, and body weight

Authors/Year of Publication	Group	HbA1c (%)		FBG (mmol/L)		Weight (kg)	
		Baseline	Changes	Baseline	Changes	Baseline	Changes
Hollander/1998	Or + hd	8.05 ± 0.98	- 0.28 ± 0.09*	8.85 ± 1.68	- 1.39 ± 0.22*	99.6 ± 14.5	- 6.19 ± 0.5*
	Pl + hd	8.2 ± 1.07	- 0.18 ± 0.11	9.09 ± 1.87	+ 0.54 ± 0.15*	99.7 ± 15.4	- 4.31 ± 0.57*
Miles/2002	Or + hd	8.87 ± 0.07	- 0.75 ± 0.08*	11.6 ± 0.2	- 2.0 ± 0.2*	102.1 ± 1.1	- 4.7 ± 0.3*
	Pl + hd	8.79 ± 0.07	- 0.41 ± 0.08	11.1 ± 0.2	- 0.7 ± 0.2	101.1 ± 1.0	- 1.8 ± 0.3
Berne/2005	Or + hd	7.6 ± 0.8	- 1.1*	11.2 ± 2.6	- 1.9*	95.3 ± 12.6	- 5%*
	Pl + hd	7.6 ± 0.8	- 0.22	10.9 ± 2.5	- 0.26	95.7	- 1.8 %
Lindgarde/2000	Or + hd	8.6 ± 1.1	- 0.9 ± 1.3*	10.95 ± 2.93	- 1.7 ± 2.1*	99.4 ± 17.5	- 6.3 ± 5.3*
	Pl + hd	8.6 ± 1.2	- 0.4 ± 1.5	10.95 ± 3.17	- 0.9 ± 2.8	98.4 ± 18.5	- 4.6 ± 5.7
Halpern/2003	Or + hd	8.37 ± 0.11	- 0.61 ± 0.15*	11.05 ± 0.27	- 1.00 ± 0.34*	89.7 ± 2.6	- 4.24 ± 0.23*
	Pl + hd	8.49 ± 0.11	- 0.22 ± 0.14	11.50 ± 0.26	- 0.01 ± 0.30*	89.5 ± 2.9	- 2.58 ± 1.46*
Guy-Grand/2004	Or + hd	7.6 ± 0.1	- 0.54 ± 0.10*	9.9 ± 0.2	- 1.39 ± 0.22*	94.3 ± 1.4	- 3.9 ± 0.4*
	Pl + hd	7.7 ± 0.1	- 0.18 ± 0.09	10.6 ± 0.3	- 0.50 ± 0.24	91.3 ± 1.3	- 1.3 ± 0.3
Shi/2005	Or + hd	7.3 ± 0.7	- 1*	8.1 ± 1.6	- 1.3*	79.4 ± 10.8	- 5.4*
	Pl +hd	7.3 ± 0.6	- 0.6	8.0 ± 1.5	- 0.5	78.7 ± 11.3	- 2.4
Kuo/2006	Or + md	9.8 ± 0.02	- 1.7 ± 0.01*	11.2 ± 0.4	- 3.40 ± 0.34*	76.8 ± 2.1	- 2.5 ± 0.6*
	Pl + md	9.6 ± 0.01	- 0.2 ± 0.01	12.1 ± 0.6	- 0.90 ± 0.12	78.3 ± 3.2	- 0.4 ± 0.3
Derosa/2012	Or + hd	8.4 ± 1.4	- 1.4 ± 0.9	7.6 ± 0.9	- 0.8 ± 0.3	94.5 ± 9.6	- 9.5 ± 3.7*
	Pl + hd	8.2 ± 1.3	- 0.3 ± 0.4	7.4 ± 0.8	- 0.7 ± 0.3	91. 7 ± 8.7	- 2.6 ± 0.9

Data are means ± SD

* Significant difference $p < 0.05$ between Or vs Pl group

Or, orlistat 360 mg/day; Pl, placebo; hd, hypocaloric diet; md, moderate-calorie, diet; HbA1c, glycated hemoglobin; FPG, fasting plasma glucose

Although orlistat is a potent agent, the administration of orlistat should be as an adjuvant to lifestyle intervention in the form of reduced caloric intake and physical activity for maximal efficacy.

Strength and limitation

According to our study, this systematic review can be developed further, considering that the number of short-term studies is less than long-term studies. The findings of this study suggest that orlistat is effective in improving glycemic control and reducing body weight in overweight or obese T2DM patients. The disadvantage of this study was that there was limited study of short-term orlistat administration as adjuvant therapy on type 2 diabetes mellitus patients with overweight or obesity, so that the effect of orlistat in the short-term use to reduce glycemic control was not convincing. The study did not consider the impact of other factors, such as lifestyle interventions or other medications, on the effectiveness of orlistat in improving glycemic control and weight loss. For long-term therapy, orlistat improved not only glycemic control, but also body weight, where normal BMI was one goal of the therapies for type 2 diabetes mellitus.

CONCLUSION

Orlistat has an effect on improving glycemic control and weight loss in overweight or obese patients with T2DM. Orlistat can significantly reduce weight both in long- and short-term administration. Based on the results of these studies, orlistat can be considered as an adjuvant therapy to lifestyle intervention in overweight or obese patients with T2DM.

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Conflict of interest

None.

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None.

Author contribution

LD and TN-conseptual idea,TN-study design, CFA-collected data, BIP, ARDJ and ANP- analysis data. LD was write the manuscript

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Review Article

PRE-OPERATIVE HORMONAL ADMINISTRATION IN HYPOSPADIAS PATIENTS UNDERGOING URETHROPLASTY

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ABSTRACT

The gold standard of treatment for hypospadias patients is reconstructive surgery. The result and post-operative complication of urethroplasty were affected by glans diameter and penile length. Pre-operative testosterone administration, both parenterally or topically, has become one of the main interests to increase the size and diameter of penis to minimize post-operative complications. However, there has not been enough evidence to justify this recommendation. Therefore, we aimed to perform a systematic review and meta-analysis to evaluate the role of pre-operative testosterone to prevent postoperative complications after urethroplasty in hypospadias patients. Online databases of Medline, Scopus and Embase were searched until October 2021 to identify RCT studies evaluating the effect of testosterone hormone therapy in reducing post-operative complication on hypospadias patient undergoing urethroplasty. Data analysis was performed using RevMan 5.4. A total of 4 RCTs were included in the analysis of this study with the total of 211 patients. Pre-operative testosterone hormonal therapy significantly reduced the overall complications group (OR=0.17; 95% CI=0.04, 0.77; p=0.02), post-operative urethrocutaneous fistula (OR=0.4, 95% CI=0.19, 0.83, p=0.01). Finally, there was no significant effect on the incidence of dehiscence and meatal stenosis with OR of 0.59, 95% CI=0.23, 1.54, p = 0.28, and 0.277; 95% CI=0.04, 1.65; p=0.16, respectively. Pre-operative testosterone hormonal therapy could reduce overall complication and urethrocutaneous fistula in hypospadias patients undergoing urethroplasty.

***Keywords**: Urethroplasty; hypospadia; hormonal therapy; health risk*

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Highlights:

1. The gold standard therapy of hypospadias is reconstructive surgery.
2. Glans diameter and penile length are factors influencing urethroplasty outcomes.
3. Many studies are being conducted to investigate various methods of increasing the size and diameter of the penis prior to the operation in order to reduce complications.
4. Preoperative testosterone hormonal therapy is able to reduce overall complication and urethrocutaneous fistula in hypospadias patient undergoing urethroplasty.

INTRODUCTION

Hypospadias is a congenital anomaly represented by an abnormal location of the urethra, which is located ventrally. The position may be located in the shaft, glans, scrotum, or perineum. It is one of the most common congenital anomalies in the field of Urology, with an incidence rate of 0.3 to 0.7% per birth (Yu et al. 2019). A large-scale national population survey in The United States of America (USA) showed that there was an increase in hypospadias prevalence from 0.2% to 0.4% between 1970 and 1993 (Springer et al. 2016). The gold standard of treatment for hypospadias patients is reconstructive surgery to form a penis with an external urethral meatus located as close as possible to the tip of penis. The penis must also be able to erect in a straight position, resembling a normal circumcised penis as close as possible (Costa et al. 2021).

Hypospadias patients are recommended to undergo an operation before the age of 18 months, because a person would have already recognized his genital organs at that age (Kaya & Radmayr 2014). The ideal timing for surgery is between the ages of 6 and 12 months to reduce psychological stress and anxiety due to an invasive procedure (Perlmutter et al. 2006). With the improvement of perioperative care for *infantas* and instrumentation technology, the age of recommendation for reconstructive surgery continues to decrease. Several surgeons even suggested an intervention between the age of 4 to 6 months with excellent outcomes. The diameter of glans and penile length are independent factors affecting urethroplasty results. Mature glans will ease the technical aspect of repair procedure, resulting in fewer postoperative complications (Bahadir et al. 2016).

Age and microphallus as a comorbid are the main factors causing difficulties during the procedure due to a small surgical field. Therefore, many studies are investigating various methods to increase the size and diameter of the penis before the operation to reduce complications. Preoperative testosterone and other hormonal therapies administration, both parenterally or topically, has become sone of the main interests of research due to its impact on the complication reduction (Krishnan et al. 2016). Several urologists have already recommended this method in patients with a small penis. Larger penis size is beneficial to allow for easier correction with a lower risk of complications (Kaya et al. 2008). However, as of the writing of this study, there has not been enough evidence to justify this recommendation.

We aimed to perform a systematic review and meta-analysis to evaluate the role of pre-operative hormonal therapy, including testosterone and its derivatives to prevent post-operative complications after urethroplasty in hypospadias patients.

MATERIALS AND METHODS

Study eligibility

The framework of PICOS which consisted of population (P), intervention (I), comparator (C), and outcome (O) was used in the framework of this study. Individual studies to be included in this meta-analysis must meet several criteria which the studies must be randomized controlled trials, the subjects were hypospadias patient undergoing urethroplasty with or without pre-operative testosterone, and the study has to contain 2 arms or more. The studies were excluded if it was case report, conference abstract, reviews, letters, or observational studies.

Search strategy and selection of studies

This study was a systematic review and meta-analysis. The subjects were pediatric patients with hypospadia. Comprehensive literature search was conducted from several databases including MEDLINE, EMBASE, and Scopus. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guideline was implemented in this study. Several keywords used in study included hypospadias, hypospadias repair, urethroplasty, testosterone, hormonal therapy, and hormone. The search was limited to human and male. The studies which were published until October 2021 were included. There were two independent reviewers who screened the study. Any contradictory and disagreement were resolved with the involvement of the third reviewers.

Data collection and risk of bias assessment

Extraction of data was conducted by the reviewers. The data were extracted into demographic data, study design, age, type of surgery, intervention protocol, serum testosterone, penile length, penile circumference, glans width, and time to procedure. Several outcomes that were evaluated were extracted into overall complication, *urethrocutaneous* fistula, wound dehiscence, and meatal stenosis incidence. The evaluation of risk of bias was also performed. For the assessment of RCTs, the Cochrane RoB2 tool was used.

Statistical analysis

The current meta-analysis was using RevMan 5.4 (Cochrane Collaboration, Oxford, UK). The studies included in this meta-analysis was only RCTs. Odds Ratios (OR) with 95% Confidence Interval was used to pool the estimation of the outcome. Mantel-Haenszel fixed-effects model was used to combine the trials if heterogeneity was low or less than 50% while Mantel-Haenszel random-effects model was selected if the heterogeneity test between studies was low or more than 50%. Statistical heterogeneity was assessed using I^2 test. The classification of I^2 test were 25%, 50%, and 75% which showed the inconsistency in low, moderate and high levels, respectively.

RESULTS

Comprehensive literature search was performed in several databases including Medline, Scopus and Embase using the search strategy. There were 311 articles identified. Through manual duplication exclusion process and using Mendeley software, 88

duplicated articles were removed. Afterwards, screening process was performed in 223 studies with title and abstract screening for each study.

On the first screening, we excluded a total of 209 articles and continue to evaluate with full-text reading of 14 articles. From 14 full-text articles, 10 articles were excluded, because the articles did not fit the eligibility criteria. On final search, a total of 4 RCT studies which fit the eligibility criteria were included in the analysis of this study.

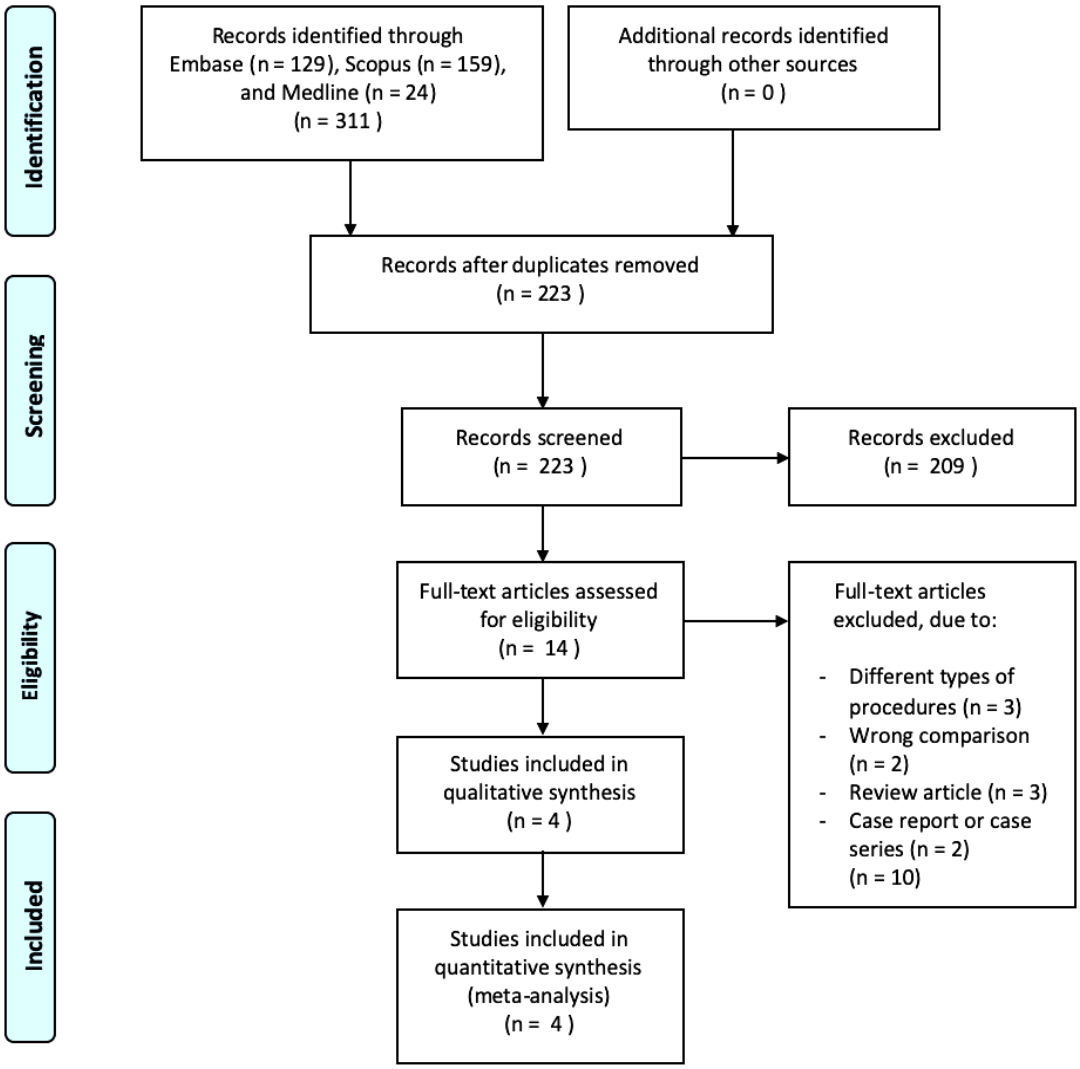

Figure 1. Screening process and identification of study summarized on PRISMA flowchart diagram

Table 1. Clinical characteristics of participants in included RCTs

Author	Type	Group	Intervention	Intervention Protocol	n	Age	Testoteron Serum (ng/mL)	Penile Length before Intervention (cm)	Penile Length after Intervention (cm)	Penile Circumference before Intervention (cm)	Penile Circumference after Intervention (cm)	Glans width before Intervention (cm)	Glans Width after Intervention (cm)	Time to Procedure	Overall Complications (n)	Complication Report
Asgari et al. 2015	RCT	Intervention	TIP Urethroplasty	Intramuscular Testoteron Enanthate	91	32.1 months	0.4	2.83	3.84	3.53	4.55	NR	NR	1 month	5	Fistula (4), Meatal Stenosis (1), Dehiscence (0), Diverticula (0)
Asgari et al. 2015 Chen et al. 2015	RCT RCT	Control	TIP Urethroplasty Flap preputial island transverse urehtroplasty / Duckett and Thiersch-Duplay combination	No Hormonal Intervention	91	28.7 months	0.37	2.81	2.88	3.51	3.57	NR	NR	1 months	12	Fistula (7), Meatal Stenosis (3), Dehiscence (1), Diverticula (1)
		Intervention		Oral Testoterone undecanoate	34	21.6 months	0.032	1.93	2.99	1.14	1.44	NR	NR	3 - 6 months	5	Fistula (2), Urethral Stricture (0), Diverticula (3), Reoperation (5)
Chen et al. 2015 Kaya et al. 2008	RCT RCT	Control	Flap preputial island transverse urehtroplasty / Duckett and Thiersch-Duplay combination TIP Urethroplasty	No Hormonal Intervention	36	24.2 months	0.056	NR	NR	NR	NR	NR	NR	3 - 6 months	15	Fistula (9), Urethral Stricture (3), Diverticula (3), Reoperation (14)
		Intervention		Transdermal DHT	37	30.8 months	NR	NR	NR	NR	NR	NR	NR	1 month	3	Fistula (0), Dehiscence (0), Scar (2), Reoperation (1), Meatal Stenosis (0)
Kaya et al. 2008 Menon et al. 2017	RCT RCT	Control	TIP Urethroplasty Snodgrass Urethroplasty	No Hormonal Intervention	38	35.1 months	NR	NR	NR	NR	NR	NR	NR	1 month	34	Fistula (2), Dehiscence (3), Scar (16), Reoperation (3), Meatal Stenosis (2)
		Intervention		Intramuscular Testoteron Enanthate	49	4.45 years	NR	3.6	4.7	3.9	5.2	1.5	1.8	3 months	5	Fistula (5), Dehiscence (7)
Menon et al. 2017	RCT	Control	Snodgrass Urethroplasty	No Hormonal Intervention	45	4.97 years	NR	4.1	NR	4.6	NR	1.6	NR	1-3 months	7	Fistula (7), Dehiscence (7)

Clinical characteristics of the trials

In this review, a total of 4 studies were included which evaluated 421 patients undergoing urethroplasty procedure (Asgari et al. 2015, Chao et al. 2017, Kaya et al. 2008, Menon et al. 2017). A total of 211 patients was allocated in group that received intervention, such as urethral hormone intervention, and urethral stricture.

Risk of bias assessment in RCT

The included RCTs were evaluated in terms of risk of bias with full-text reading and the application of RoB tools 2 by Cochrane Collaboration. In the bias domain due to randomization process, we found that majority of studies used randomization technique through computer. Thus, the risk of bias caused of randomization process was considered to be low in all studies except the study from Kaya et al. (2008) that did not explain randomization process adequately. In the domain bias due to deviation from intervention, we found that the risk of bias was considered to be low in all studies. Based on the assessment of risk of bias due to the missing outcome, we found that several concerns on the study from Chen et al. (2015), Kaya et al. (2008), Menon et al. (2017), there were no adequate information regarding the number of patients that dropped out or lost to follow up. Overall, the evaluation on 4 domains showed that the risk of bias between the studies was considered low (Figure 2).

The effect of hormonal therapy to overall complications

In terms on the outcome of overall complication, the analysis of 4 RCTs which evaluated 421 patients undergoing urethroplasty procedure using pre-operative testosterone hormonal therapy was compared with control (Asgari et al. 2015, Chao et al. 2017, Kaya et al. 2008, Menon et al. 2017). Based on the result from forest plot analysis, there were significantly fewer overall complications in groups receiving pre-operative hormonal intervention compared to control group (OR=0.17; 95% CI=0.04, 0.77; p = 0.02). In this study, analysis was conducted by using random-effects DerSimonian model due to high heterogeneity among studies (I^2 = 84%, p = 0.0004) (Figure 3).

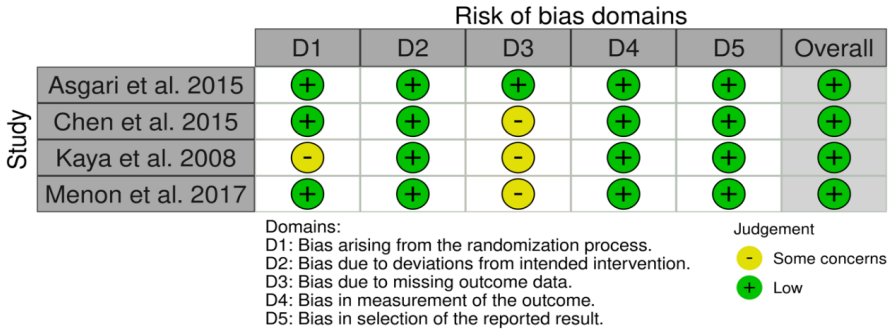

Figure 2. Risk of bias assessment using Cochrane risk of bias (RoB) tools 2

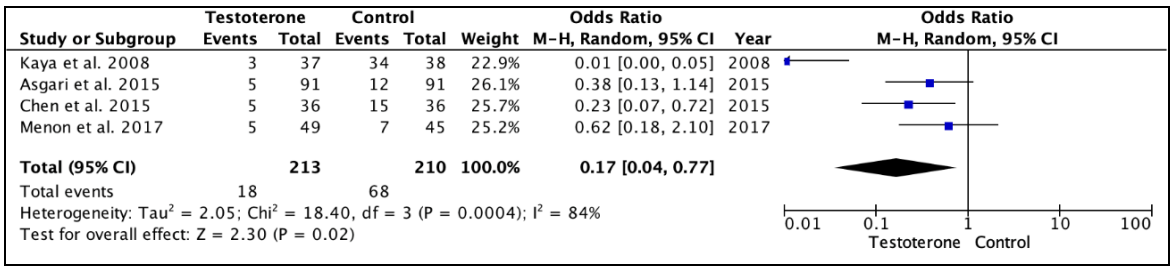

Figure 3. The result of forest plot analysis on the incidence of overall complication in groups receiving preoperative hormonal treatment compared to control

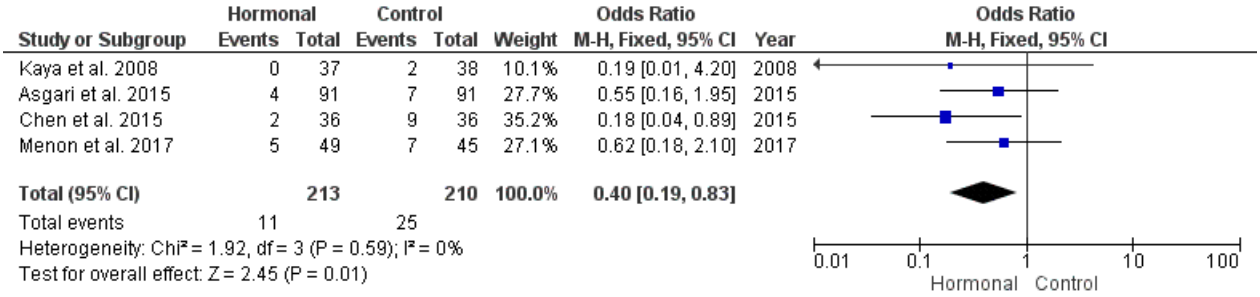

Figure 4. Forest plot analysis result on the incidence of urethrocutaneous fistula in groups receiving pre-operative hormonal therapy compared to control

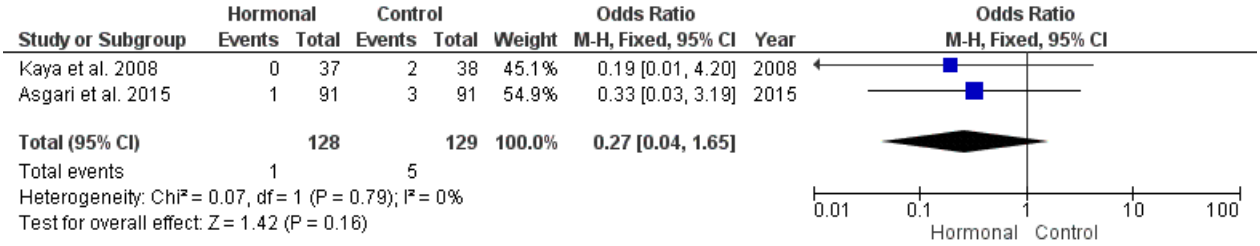

Figure 5. The analysis of forest plot on the incidence of dehiscence in groups receiving preoperative hormonal therapy compared to control

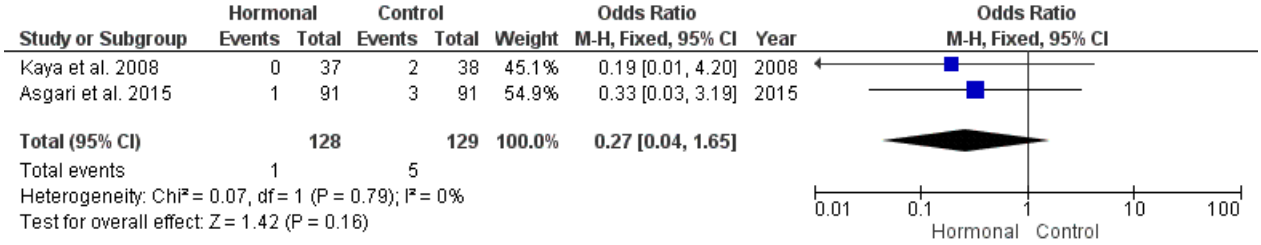

Figure 6. The analysis of forest plot on the incidence of meatal stenosis in group with preoperative hormonal therapy compared to control

The effect of hormonal therapy on the incidence of urethrocutaneous fistula

Based on data extraction that we performed, all RCTs reported the incidence of urethrocutaneous fistula. Several studies showed insignificant result on the incidence of urethrocutaneous fistula, while other studies reported the significant result. Based on final analysis, we found that hormonal therapy significantly reduced the incidence of post-operative urethrocutaneous fistula (OR=0.4, 95%C=0.19, 0.83, p=0.01). The analysis of forest plot showed that heterogeneity between included RCT studies was classified to be low with I^2 test of 0% and the heterogeneity p-value was 0.59, thus fixed-effect model was used (Figure 4).

The effect of hormonal therapy on the incidence of dehiscence

Based on the analysis of forest plot analysis in 3 RCT studies which evaluated the effect of hormonal therapy on the incidence of dehiscence, there were no significant difference between the group receiving pre-operative hormonal therapy compared to without hormonal therapy (OR=0.59, 95% CI=0.23, 1.54, p=0.28). Besides, the studies had good heterogeneity with p value 0.45 and I^2 of 0% (Figure 5).

The effect of hormonal therapy on the incidence of meatal stenosis

There were 2 RCTs which evaluated the effect of hormonal therapy on the incidence of meatal stenosis. Based on the pooled analysis in 257 participants which were allocated in hormonal therapy group (n=128) and control group (n=129), hormonal therapy did not comprise significant effect on the incidence of meatal stenosis reduction (OR=0.277; 95% CI=0.04, 1.65; p=0.16). Heterogeneity between RCT studies was considered low with I^2 test of 0% and heterogeneity p value of 0.79. Thus, the analysis was using fixed-effect model (Figure 6).

DISCUSSION

One most common post-operative complication of hypospadias is suspected to be caused by surgical difficulties due to penis size. This factor becomes a burden for a surgeon to perform corrective surgery well. This difficulty may also lead to several post-operative complications, causing morbidity and a lower quality of life (Lucas et al. 2020, Ru et al. 2021). Based on these considerations, several studies have been investigating several methods to increase penis size before surgery.

Based on the mechanism of action of the hormone on penile growth and development, several studies suggested the use of pre-operative testosterone as a stimulator for increasing penis size. Several surgeons believed that pre-operative androgen administration can facilitate penile reconstruction to achieve excellent results and outcomes (Mohammadipour et al. 2020). Several studies suggested that small penis size should be considered as an independent risk factor for post-operative complications (Bush et al. 2015, Wong & Braga 2015). Before evaluating the role of testosterone in reducing post-operative complications the role of testosterone in increasing penis size has to be clear. Satav et al. (2015) discovered that both parenteral and topical testosterone may achieve the desired penis size compared to pre-testosterone administration. Previous systematic reviews on a similar topic resulted in inconsistent and various results with a low quality of evidence (Chao et al. 2017, Kaya et al. 2008, Wright et al. 2013).

In this review, we discovered that there was a significantly lower complication rate in patients receiving the role of pre-operative hormonal therapy, including testosterone and its derivatives (OR 0.17; 95% CI 0.04, 0.77; p = 0.02) (Asgari et al. 2015, Chen et al. 2015, Kaya et al. 2008, Menon et al. 2017)(S. A. Asgari et al., 2015; C Chen et al., 2015b; Kaya et al., 2008b; Menon et al., 2017b). A larger penis size allows for an easier surgical correction. Androgen stimulation would increase tissue size with high vascularization, which could also facilitate tissue healing. (Bastos et al. (2011) discovered that the administration of 1% testosterone ointment could increase the volume and density of blood vessels. This increase is most beneficial in patients with microphallus or who had a previous unsatisfactory outcome (Ahmad et al. 2011). Aside from the intraoperative factors, the effects would also decrease the post-operative formation of scar tissue (Bahadir et al. 2016).

We also discovered that pre-operative hormonal therapy administration can decrease the incidence of urethrocutaneous fistula to 60% (OR 0.4, 95% CI 0.19, 0.83, p = 0.01), but not significant in meatal stenosis incidence (p = 0.16) and dehiscence (p = 0.28) (Asgari et al. 2015, Chen et al. 2015, Kaya et al. 2008, Menon et al. 2017). There are some negative effects of testosterone reported by studies. Several studies inhibitory effects of androgen on wound healing and increasing inflammation on tissue. Some patients also complained of penile discomfort, unwanted erection, pubic hair growth, and pigmentation (Gorduza et al. 2011).

Some studies also reported that a long-term administration of testosterone for 3 months or more could increase the rate of post-operative complications instead (Netto et al. 2013). The difference in results was due to the lack of consensus and standard regarding the type, dose, and route of administration for pre-operative testosterone in hypospadias management. Positive results from this systematic review was expected to be one of the basis for creating a recommendation regarding the pre-operative testosterone administration (Asgari et al. 2015, Kaya & Radmayr 2014). Other studies also evaluated the role of endogenous hormones, such as estradiol and B-HCG, which could also increase penis size. However, the number of studies evaluating the role of these hormones was limited (Krishnan et al. 2016). There are several limitations to this systematic review.

Strength and limitation

Due to the limited number of published RCTs, the evaluation of hormonal administration was performed on all routes of administration. There is also one study that evaluated *dihydrotestosterone* as intervention. Different types of urethroplasty were also performed by the included studies. As more published studies become available, a sub-group analysis evaluating each type of hormonal therapy can be performed. Adverse events of the intervention should also be analyzed quantitatively in the future. These side effects might affect the patient's quality of life; thus, its potential occurrence should be considered to conduct a treatment strategy.

CONCLUSION

Pre-operative hormonal therapy administration including testosterone and its derivatives may decrease the postoperative complication rate in hypospadias patients, as indicated by a lower incidence of urethrocutaneus fistula and overall complications. Further studies with a larger sample and randomized design assessing different sub-groups of hormonal therapy are required.

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Conflict of interest

None.

Funding disclosure

None.

Author contribution

All auhtors were conseptual idea, study design, collected data, BIP, ARDJ and ANP- analysis data. LD was write the manuscript

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Review Article

RADIOLOGICAL ASPECTS OF HR-CT SCAN ON TEMPORAL BONE

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ABSTRACT

The high-resolution computed tomography (HRCT) scan used in the 1980s offers a distinct advantage in interpreting images of the temporal bone. To obtain the right image reconstruction and to provide meaningful information, a certain degree of tilt is required, so that radiologists and clinicians can get more real imaging information on structural abnormalities in the temporal bone and its soft tissue constituents. The technique or protocol in HRCT of the temporal bone becomes an essential primary aspect in presenting the analyzed structure, the assessment of the small form of the auditory bones, the soft tissue of the inner ear and the cranial nerves that pass through the temporal bone structure is much easier to analyze with the help of reconstruction according to the HRCT protocol for temporal bone, yet the MRI is preferable for soft tissue evaluation. In the end, the standard structure, congenital abnormalities and pathological problems in the temporal bone structure can be identified and informed to the clinician as a step to determine further treatment.

***Keywords**: Radiological aspects; HR-CT Scan; temporal bone; congenital abnormality; illness*

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Highlights:

1. High-resolution computed tomography (HRCT) scan has advantage for best image reconstruction and informative in interpreting images of the temporal bone.
2. The essential primary aspect in HRCT of the temporal bone scan is technique and protocol.

INTRODUCTION

High-resolution computed tomography (HRCT) is a type of commonly used CT scan with a special technique to increase the resolution or sharpness of the CT image, with a cut thickness of about 0.625-1.25 mm reconstructed with a high algorithm using a bone algorithm. Using a scan time of 0.5-1 second, with a power of 120 kV, 100-200 mAs is required with collimation of 1.5-3 mm, a matrix size of 768 x 768 or its largest resulting in better imaging (Shaffer et al. 1980; Torizuka and Hayakawa 1992).

OVERVIEW

HR CT technique of the temporal bone

Currently, there are two techniques for taking CT of the temporal bone, namely MSCT (Multi-Slice CT) which is commonly used, and CBCT (Cone Beam CT) which have started to take over the role of MSCT. CBCT uses a rotating gantry, where the x-ray tube and the detector touch. The conical x-ray beam is directed directly through the middle portion of the temporal bone towards the two-dimensional detector. Since CBCT uses a two dimensional FOV detector, only one 360 degrees rotation of the gantry produces 3-dimensional data. 110 kV, ± 140 mAs, field of view 15x5 cm high resolution, slice thickness 0.15 mm, scan time 40 s is a parameter that becomes a reference protocol for scanning with CBCT (McCollough & Zink 1999).

The resulting MSCT image is obtained from a single image plane using a multi-slice detector. Patients only need to lie in the gantry without a specific marker and then the radiologist just follow the image parameters according to the machine with the following parameters: 120 kV, 250 mAs, collimation 0.5 mm or 0.625 mm and scan time 1.0 s. After completing the process, the data was processed almost similarly to CBCT, reconstructed with 1 mm thickness using ultra high-resolution mode. The technician was instructed to make a parallel reconstruction with the semicircular lateral canal to produce an axial cut, while the coronal

cut was reconstructed right on the peripendicular perpendicular to the axial. The most anterior coronal section is directly anterior to the geniculate ganglion of the facial nerve. This procedure is applied to both the right and left temporal bones, using a window depth of 4000 HU and a window level of 200 HU, which actually depends on the convenience of the radiologist which makes it easier to see and analyze the stapes and suspensory ligaments in the middle ear cavity (McCollough & Zink 1999).

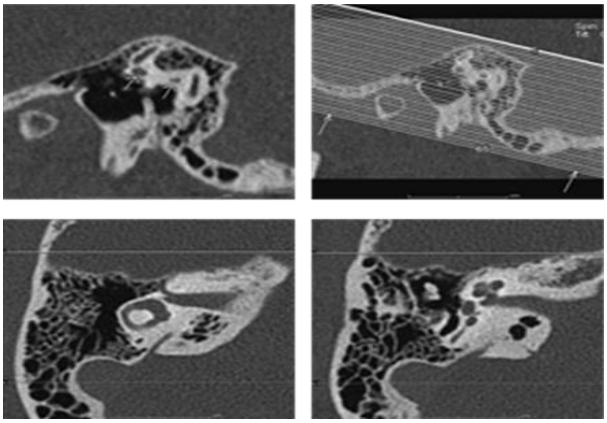

Figure 1. Creation of standard axial and coronal CT scan data of temporal bones

Note: (a) after the data source has been obtained in three dimensions (3D) on the scanner console, image is sagittal and then scrolled to obtain an image of the anterior and posterior limb canal semicircularis lateralis (short white arrows). (b) make axial cuts by setting overlapping parallel lines with a distance of 0.1 mm. (c) if the axial reconstruction is correct, it will reveal the entrance of the lateral semicircularis canal. (d) when scrolled, it showed cochlear fossette, modiolus and footplate stapes that are outlined.

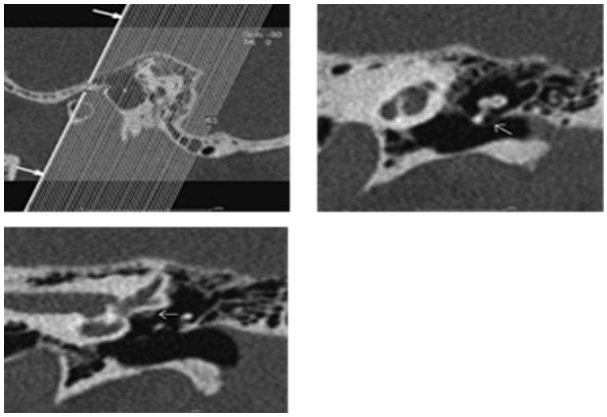

Figure 2. The coronal reconstruction is made in the same steps as the axial cut, so that the finished coronal cut could show Prussak's space (short white arrow) and facial nerve canal (white arrow in the last image) clearly

Temporal bone anatomy

The temporal bone is located on the lower side of the cranium base. It is located on the lateral side of the temporal lobe of the cerebral, consisting of five bones that compose it, among others: squamous, petrous, mastoid, tympanic, styloid processus. Inside the temporal bone, there are several essential ear structures, namely middle and inner ear. The lower part of the temporal bone is also a part of the joint with the mandibular bone which forms the temporomandibular joint (Graham et al. 2000).

Ear anatomy

The ear consists of three parts; outer, middle and inside. This section detects, delivers and converts sound signals into electrical stimuli that are transmitted by afferent auditory nerve fibers to the central nervous system.

Outer ear

The outer ear has a canal ± 3 cm in length, where 1/3 laterally is on the cartilage, while 2/3 inside is hard bone. This canal narrows and curves to protect the tympanic membrane from foreign objects and direct trauma. Innervated by n. auriculotemporal (n. trigemine), n. plexus cervicalis, n. vagus and n.facialis. In acoustic neurinoma patients, cough reflex originating from n. vagus disappears (Hitselberger's sign) (Park et al. 2000).

Middle ear

Middle ear consists of several components, namely cavum tympanic, temporal bone pneumatic system, and Eustachian tube. The tympanic cavity and the pneumatic system are aerated from eustachian tube can open in the nasopharynx. The outer ear canal and tympanic cavity are separated by eardrum which forms a 55 degree angle with the ear canal on the hard bone (Swartz & Harnsberger 1998).

The middle ear cavity is divided into three parts based on the level of the tympanic membrane. The level above tympanic membrane consists of the epitympanic recess of the attic with the head of malleus os, the incus body and the ligament and muscle folds which then pocket together with the chorda tympani. The level of the tympanic membrane consists of the mesotympanum, while the level below the tympani membrane consists of the hypotympanic recess. Between epi and mesotympanic anatomically, some constructs allow retention of secretions during inflammation and lack of aeration in the attic. This causes chronic epitympanitis and cholesteatoma. The pneumatization of temporal bone varies widely. The pneumatization system is well-developed that extends to both occipital and zygomatic arch regions. Formation of the mastoid process begins after birth between the second or the fifth year, and the process is complete at the age of 6-12 years. In this area, aeration blockage during childhood will result in repeated inflammation of the middle ear. This inflammation can have more severe complications in the future (Hasso et al. 1996, Park et al. 2000).

Ossicles

Originating from the inner surface of the tympanic membrane to the oval window, there are interconnected bony bones that can still move, called as ossicles.

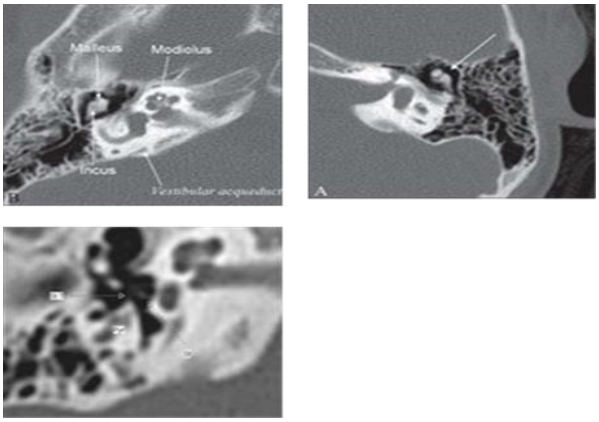

Figure 3. (a) Left axial CT scan temporal showing incus and malleus. (b) right axial CT scan cut temporal showing association with modiolus and vestibular aquaduct (white arrow). (c) bone stapes (white arrows)

Eustachian tube

The Eustachian tube is a link between middle ear and nasopharynx. Its function is to balance the pressure passing through tympanic membrane. Contraction of tensor veli palatine and salpingopharyngeus outer side of tympanic cavity dilates and opens the eustachian tube.

Inner ear innervation

The horizontal portion of the facial nerve passes through the tympanic cavity in the labyrinth wall in the bony canal just superior to the footplate of the stapes—Chorda tympani, branches that return from the facial nerve before exiting through the stylomastoid foramen. The chorda tympani enters the middle ear after leaving the bony canal and passes through the medial side of the neck of the malleus bone, then fuses to the carotid wall. The chorda tympani supplies the submandibular and sublingual glands as well as the anterior 2/3 of the tongue. Sensory information about the tympanic canal (middle ear) is carried by multiple nerves that form the tympanic plexus at the promontory in the medial wall.

Inner ear

The inner ear, also known as labyrinthine cavity, functions to transmit sound to the central nervous system as well as assist balance. Auditory transduction, converting mechanical energy into electrochemical, occurs in the cavity of the labyrinth. The labyrinth cavity is essentially made of a labyrinthine membrane surrounded by bones. Labyrinth bone is a series of bony cavities inside the temporal bone. The labyrinth membrane is the membrane sacs and channels that are connected inside the labyrinth bone. The labyrinthine membrane is surrounded by perilymph and contains endolymph. The labyrinthine membrane also has cochlear, vestibular and semicircular components.

What to report on the examination of the temporal bone?

The format of reporting the results of the HRCT examination of the temporal bone has been widely discussed in articles circulating at this time. We tried to present a quite complete format, including structural assessments and possible things that clinicians need, including identity, date of examination, previous examination for comparison, clinical indication for HRCT examination of temporal bone (Graham et al. 2000, McCollough & Zink 1999).

At each examination of the temporal bones, either right or left were assessed: External auditory canal, mastoid complex, middle ear covering; whether the bone ossicles and pneumatization were normal, the tympani tegmen and scutum were normal, the oval and round window were standard, the inner ear included cochlea, vestibule, semicircular canal and internal auditory canal were normal, the vestibular aquaduct was not enlarged, the passage and caliber of the facial nerves, including the mastoid pars were normal, the internal carotid artery and jugular veins travelled normally (McCollough & Zink 1999, Swartz & Harnsberger 1998).

Congenital abnormalities

Congenital abnormalities of the outer ear

Earlobe

Malformation of the earlobe can affect the size, shape, position and orientation of the pinna (auricle). The complete absence of pinna (anotia) may occur. Various classification schemes have been proposed to assess the severity or deformity (Weerda 1988).

Table 1. Classification of ear lobe malformations

Dysplasia class	Characteristic
Class I (mild malformation)	Most of the structures resemble normal ear lobe.
Class II (moderate malformation)	Some structures are still normal, when the operation requires additional skin or cartilage tissue
Class III (severe malformations)	There was no visible structure of the normal earlobe

External auricular canal

Failure to recanalize the first cleft tissue can lead to malformation of external auricular canal (Ko¨sling et al. 2009, Mayer et al. 1997). The absence of a meatal opening below the tragus indicates complete aural attresia. Attresia or incomplete stenosis should be

suspicious if the pinna is abnormal and the external auricular canal diameter is less than 4 mm or the tympanic membrane cannot be visualized (Chandrasekhar et al. 1995).

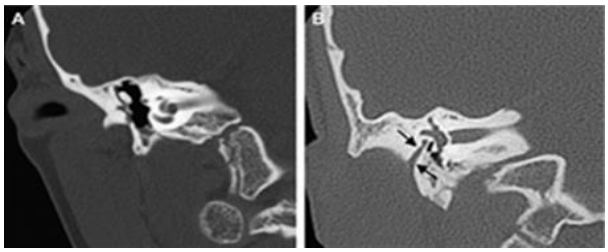

Figure 4. (a) coronal cut CT scan of the right temporal bone at the level of the cochlea showing total atresia of the external auditory canal. (b) coronal CT scan of the right temporal more posteriorly at the level of the vestibule showing a short and shallow short and shallow facial nerve segment

First branch cleft anomaly

This occurs as a result of the abnormal ectodermal closure of the gap. It usually occurs along the baseline of the auricular canal external to the submental area. These disorders can occur as preauricular cysts, sinus or recurrent external otitis (Yalcin et al. 2003).

Congenital cholesteatoma

Congenital cholesteatoma occurs more frequently in the middle ear. Children with attresia canalis auricular externa can develop cholesteatoma (either primary or secondary) at the site of stenosis or deep into the attretic plate (Johnson et al. 1983).

Congenital abnormalities of the middle ear

Malleus

These include aplasia, fixation of incudomaleolar joint, fusion of the malleus head to the long process of the incus head and stapes (triple bony union). A rare malleus anomaly is congenital fixation. From the head of the malleus to the lateral epitympanic wall called as malleus bar.

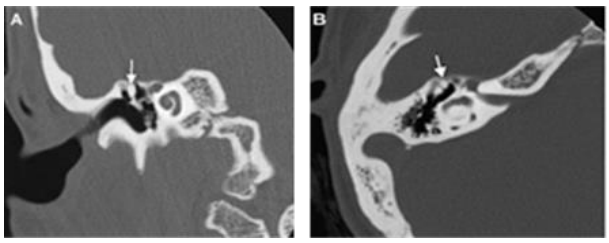

Figure 5. (a) coronal CT scan of the right temporal showing attached malleolus bone fixed superolateral to the epitympanic wall. (b) same axial temporal CT scan section

Incus

Abnormalities including aplasia, long processus deformity, fusion of the short processus incus to the lateral semicircular canal and fibrous fusion of the incudostapedial joint or joint absence.

Stapes

These included absent stapes superstructure, aplasia, stapes head abnormalities, monopod stapes, fixation of the stapes head to the promontorium, and footplate fixation.

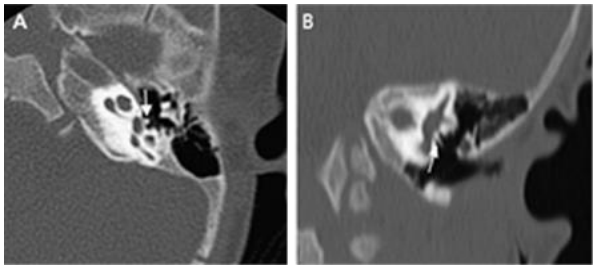

Figure 6. monopod stapes. (a) axial slice and (b) coronal reformat section of tempo CT scan left ral which shows single stapes called as monopod stapes

Other anomalies in the middle ear included persistence of the stapedial artery, absence of the stapedius muscle. Congenital cholesteatoma is identified as a whitish mass behind an intact tympanic membrane (Johnson et al. 1983).

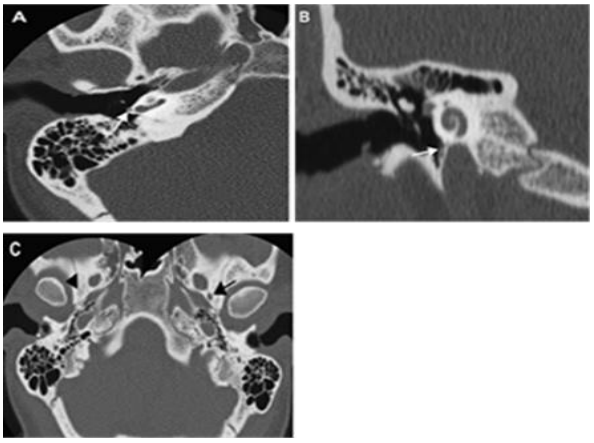

Figure 7. Persistent stapedial artery

Note: (a) axial section and (b) coronal section CT scan of the right temporal showing the canalis still at the level of the promontory cochlea indicated by arrows. (c) on further examination, it was found that there was no normal foramen spinosum on the right side which was shown by the arrowhead when compared to the left side.

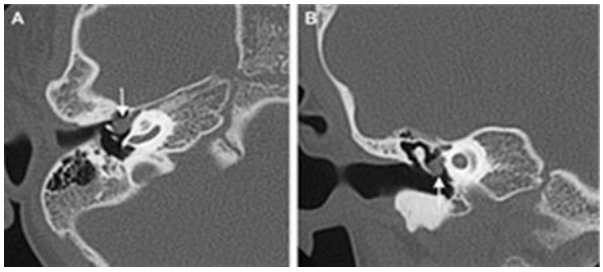

Figure 8. Congenital cholesteatoma. (a) axial section and (b) coronal section CT scan temporal showing rounded soft tissue with a flat edge in the middle ear space

The tympani cavity

Congenital abnormalities were hypoplastic, aplastic or extracavitation, and it was an extra cavity in an improper place.

Mastoid pneumatization

There might be normal, reduced or even non-pneumatized formation of the mastoid.

Congenital abnormalities of the inner ear

Malformation of the inner ear occured as a result of disruption at embryogenesis stage. A newer classification had been proposed by Sennaroglu and colleagues (Sennaroglu & Saatci 2002).

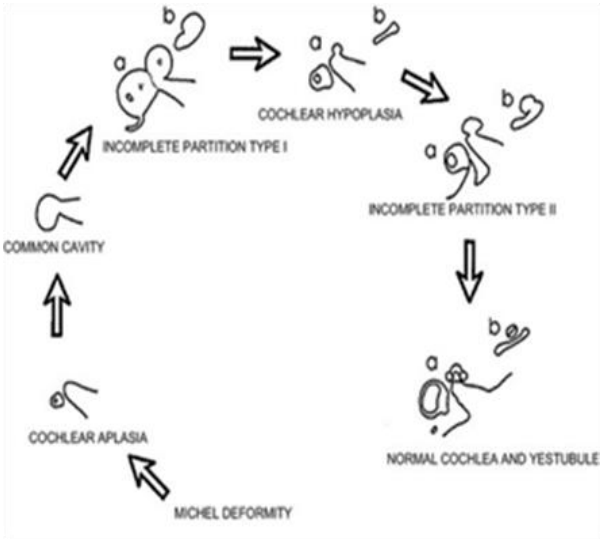

Figure 9. A schematic of a different degree to the development of the inner ear wherein the letters a and b represent successive stages that pass through the internal auditory canal and round window

The basis of this classification was embryological growth, but it also distinguished between classic Mondini and pseudo-Mondini abnormalities.

Michel deformity or labyrinthine aplasia

In this malformation, there was a complete labyrinthine aplasia to the point, where cochlear and vestibular elements are not formed. Due to the failure of the development of the otic vesicles to the absence of the formation of inner ear, this anomaly is described as an otocytic deformity and described as the extremely rare and the most severe inner ear abnormality (Quirk et al. 2019).

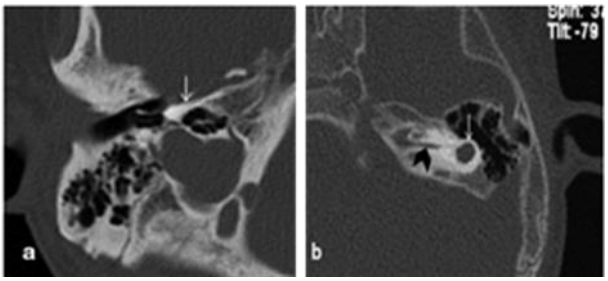

Figure 10. Michel Deformity (a) arrow shows no labyrinthine in small and dense otic capsule (b) white arrow shows rudimentary otocyst with narrowed internal auditory canal (black arrow)

This deformity occurs after the formation of the otic vesicles is complete, because the semicircular canal is the first structure to be formed from the otic vesicles. It is possible that the inner ear presents some rudimentary abnormalities of the semicircular canal. Failure to differentiate the promontory cochlear from associated labyrinthitis. A similar condition can occur after the condition of meningitis, where the formation of connective tissue and changes in the bone structure of the inner ear, including the cochlea take a place (Swartz & Harnsberger 1998).

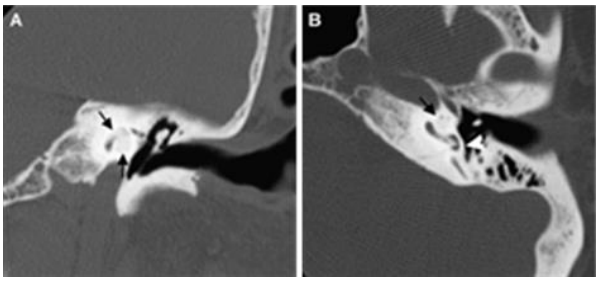

Figure 11. Labyrinthitis ossificans. (a) coronal slice and (b) axial cut CT scan of left temporal showing ossification of the middle grooveand left apical cochlea of ossification labyrinthitis (black arrow), while the head of the white arrow indicates a normal cochlear promontorium Labirinitis

Cochlear aplasia

This malformation takes the form of the complete absence of ochlea. The vestibule, semi-circular canal and internal auditory canal are still present and normal, and sometimes hypoplastic.

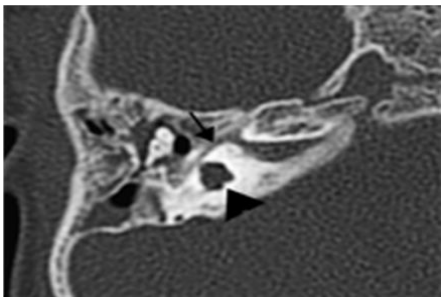

Figure 12. Cochlear aplasia. Shaped axial CT scan of right temporal showing absence of cochlea, arrowhead showing dysplastic right vestibule. There was also a mild right facial nerve abnormality indicated by the arrow activation mechanism

Common cavity deformity

Common cavity malformations in the form of cystic, cochlear fusion, vestibule and semicircularis canal including size variations. This disorder is often associated with narrowing or widening of internal auditory canal and dysplasia canalis semicircularis (Graham et al. 2000).

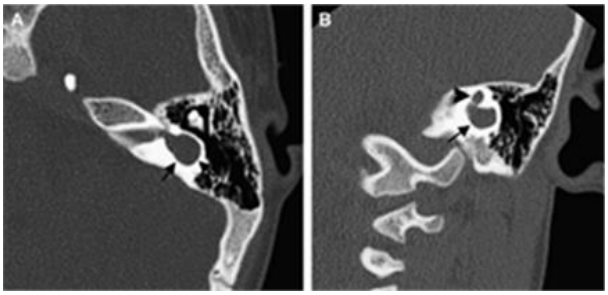

Figure 13. Common cavity malformationssowed axial section (a), and coronal section CT scan of right temporal showing incomplete structures of the vestibule, cochlea and semicircular canal (b)

IP type I: Cochleovestibular cystic malformations

Distinguished for more clearly malformations between Pseudo-Mondigi (IP I) and classic Mondigi (IP II) by Sennaroglu and colleagues in 2002.

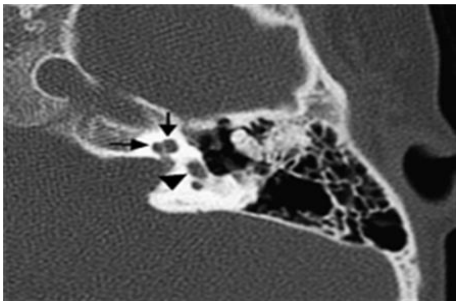

Figure 14. IP type I (number eight "8" malformation). On the axial CT scan the right temporal scan shows the cystic-shaped coclea (arrowhead) and vestibule (arrow) with no internal structures

abnormalities, the cochlear dimensions and vestibule are normal but have architectural defects in them. Cochlea is without a modiolus and looks cystic with a dilated vestibule without internal structure (Graham et al. 2000).

Cochlear hypoplasia

This deformity indicates a hypoplastic anomaly that is not even formed at all.

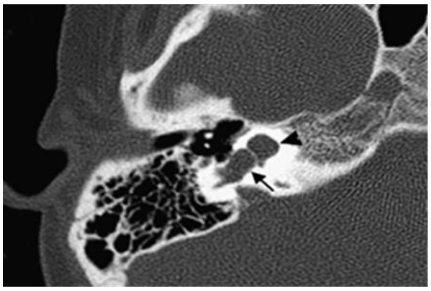

Figure 15. Cochlear hypoplasia. Axial cut CT scan of the left temporal showing cochlea that looks small (arrowhead) and vestibular (arrowhead)

IP type II: Classic mondini deformity

This malformation is in the form of a cochlea that rotates 1.5 times from the normal 2.75 turns. Interscalar defect occurs between the middle and apex rotation, where it coalesces into 1 space. Compared to IP I, type II is more common. Having a basal cochlear modiolus in type II, the incidence of meningitis is lower, and the likelihood of restoration of hearing with implantation is better (Graham et al. 2000).

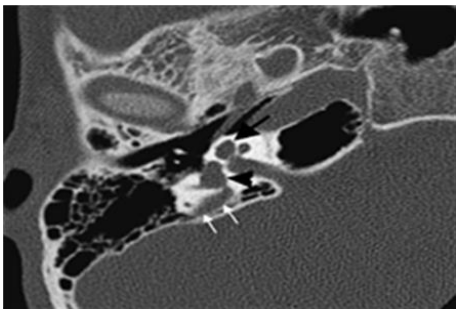

Figure 16. IP type II (Mondini malformation) Shown in the right axial CT scan of the right temporal showing the joining of the middle and apical grooves of the cochlea (black arrow). Large visible vestibule (arrowhead) and also visible widening of the vestibular aqueduct (white arrow)

Vestibular malformations

Independent vestibular abnormalities are very rare, usually co-occurring with IP I, IP II disorders and cochleovestibular hypoplasia. Semicircularis canal malformations include; dysplasia canalis semicircuralis lateralis, common crus anomaly, and other anomalies such as CHARGE and BOR syndrome.

Dysplasia of the lateral semicircularis canal; is the most common disorder in the form of a short lateral semicircularis canal, with a wide and fused vestibule with common cavity, the superior and posterior semicircularis canals appear in normal proportions. Common crus anomaly, the posterior and superior semicircularis canals may widen and merge with the vestibule and common crus may not develop properly.

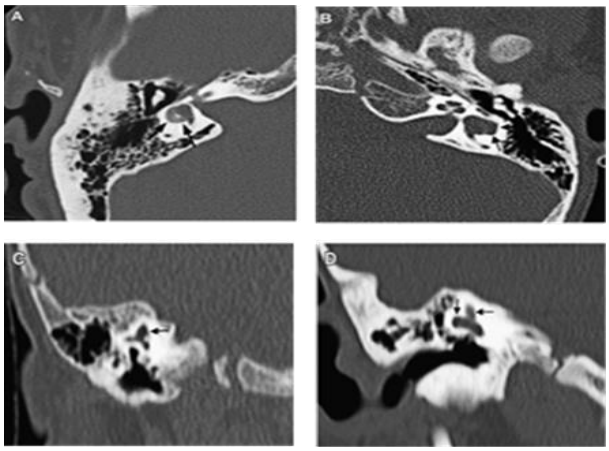

Figure 17. Dysplasia of the semisircularis superior canalis

Note: (a) axial CT scan of the right temporal showed shortening of the lateral semicirculariscanal (arrow) suspected to be associated with mild dysplasia, (b) axial CT scan of left temporal in a different patient showed left vestibular dysplasia and left lateral semicircularis canal fused to form a common common. cystic cavity (arrow), (c) coronal cut CT scan of the right temporal showing dysplastic and enlarged right semicircular common crus canalis posteriorly and superiorly (arrows), and (d) coronal cut CT scan of the right temporal showing similar abnormalities.

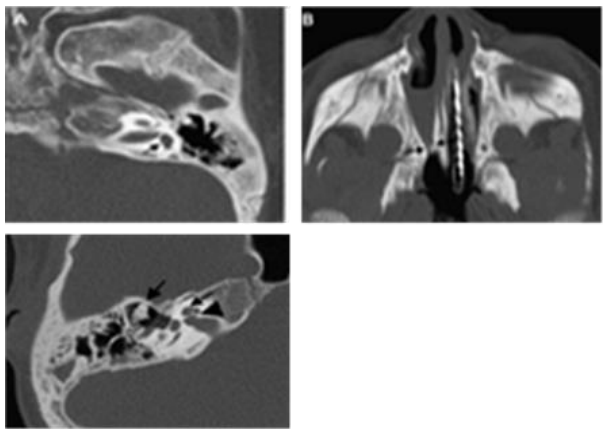

Figure 18. CHARGE syndrome

Note: (a) axial cut CT scan of the left temporal showing the absence of all semicircular canal structures with mild dysplasia of the left vestibule (arrow), (b) axial CT scan at mid-face level showing right choanal atresia, (c) Branchio- auto-renal syndrome. Axial cut CT scan of the right temporal showing hypoplastic indentation of the apical coclea (small arrow). Invisible cochlear modiolus was suspected of hypolasis (arrowhead). Also, there was a short, calcified superior ligament located more anterior to the middle ear bony bones (Propst et al. 2005)

Other semicircularis canal abnormalities; CHARGE anomaly, is the total absence of the semicircularis canal. Meanwhile, BOR syndrome is an autosomal dominant disorder affecting the EYA 1 gene on chromosome 8 which is characterized by branchial fistulas and cysts, ear abnormalities that cause deafness, kidney malformations, inner ear abnormalities including cochlear hypoplasia, lateral semicircularis canal hypoplasia and cochlear aquaduct enlargement.and vestibular (Propst et al. 2005).

Enlarged *vestubular aquaduct*

This disorder is one that often occurs in middle ear malformations. It was reported in a study that enlarged vestibular aquaducts were probably associated with cochlear malformations in 76% and vestibular malformations in about 40% in each case (McElveen et al. 1997).

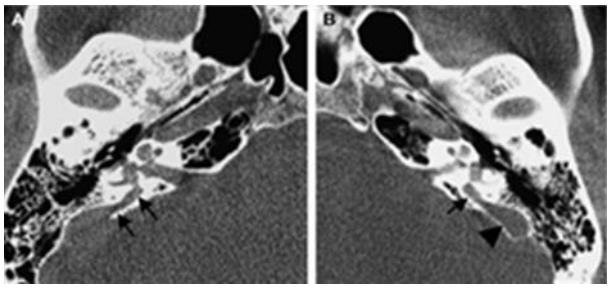

Figure 19. Syndrome of enlarged vestibular aquaduct

Note: (a) right axial section, (b) left axial section Temporal CT scan showed bilateral enlargement of the vestibular aquaduct (arrow) and prominent endolymph sac (arrowhead) (McElveen et al. 1997).

Internal auditory canalis stenosis

Rare disorder, where internal auditory canal diameter is <2 mm, and it is usually associated with narrowing of the cochlear nerve canal.

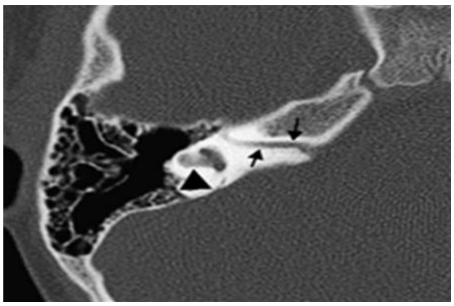

Figure 20. Refinement of the internal auditory canal. Right temporal CT scan axial cut showed narrowing (arrow). The patient also had a dysplastic vestibule and partially visible semicircularis canal (arrowhead)

Anomaly related to the X chromosome

It is a rare condition characterized by dilatation of the bulb of lateral end of the internal auditory canal. This disorder is associated with sensory deafness, and the risk of cerebrospinal fluid bursting at cochleostomy period (Phelps et al. 1991).

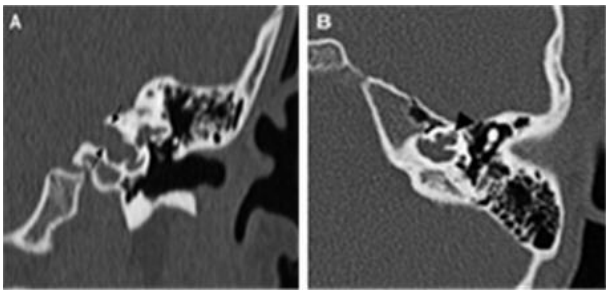

Figure 21. X chromosome anomaly

Note: (a) coronal cut, and (b) axial cut CT scan of left temporal showed abnormally dilated internal auditory canal (arrow) with dysplatic cochlea (arrowhead) accompanied by absence of cochlear modiolus

Figure of inflammation and infectious disease on CT scan of the temporal bone

Suspicion of inflammation and infectious disease in the temporal bone is the main indication for HRCT examination (Howard et al. 1990).

Outer ear

Acute otitis externa or swimmer's ear

The most common infection is characterized by pain, redness, swelling, often with conductive hearing loss when external auditory canal is obscured by this situation. These acute conditions are obstacles to CT scan examination.

Usually, the images showed things that were still normal, but it could be different if the clinical showed recurrences; or there were situations that underlied the abnormality, such as benign or malignant lesions (Swartz & Harnsberger 1998).

Chronic external otitis

CT scan imaging provides a good overview of the anatomical details behind obstruction by this chronic situation, including the extent and extent of bone erosions resulting from infection. Chronic inflammation results in subepithelial infiltration leading to fibrosis and stenosis of the external auditory canal (Hasso et al. 1989).

Malignant otitis externa

Demonstrated otorrhea and severe otalgia in both diabetic and immunocompressed patients. This condition is also related to a history of trauma. On physical examination, there is granulation tissue in the external auditory canal inferiorly, along the junction of the bone and cartilage. The infection spreads to the inferior side to the soft tissues and the temporomandibular joint (Howard et al. 1990). Malignant otitis externa has been classified according to its grade, namely Grade I: pinching of the external auditory canal with and without facial nerve paralysis, Grade II: extension to the superior, skull base osteitis, and/ or involving multiple cranial nerves, and Grade III: extension to the temporal or intracranial bone and involves the contralateral.

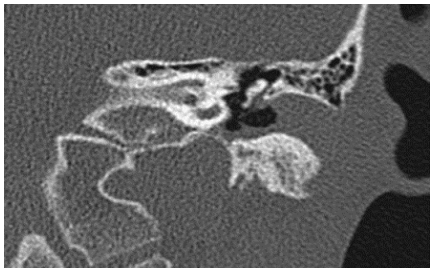

Figure 22. Left temporal CT corona-cut scan showed soft tissue filling the external auditory canal in a diabetic patient with Grade I (pinching) malignant otitis externa. Thickened tympanic membrane

Middle Ear

Acute otomastoiditis and its complications

manifested clinically as ear pain, fever, and redness of the tympani membrane. The middle ear and mastoid are generally exposed to expansion of infection from upper respiratory tract and bacteria enter through \eustachian tube. Temporal CT scan can show an image of ossicular erosion, destruction of the middle

ear which resembles an aggressive tumor accompanied by lymphadenopathy.

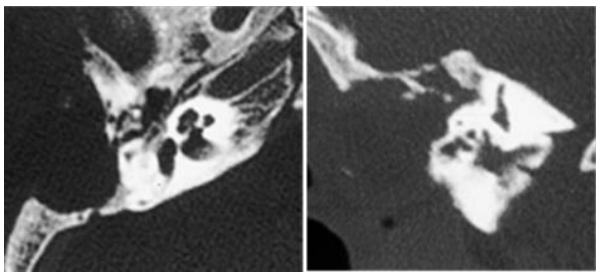

Figure 23. Tuberculous otomastoiditis with parenchymal abscess. (a) axial section (b) coronal cut CT scan right temporal showing middle ear destruction

Coalescent mastoiditis

A temporal CT scan is performed if there is suspicion of non-specific debris which is indicated by the presence of multiple air fluid levels. A temporal CT scan will show the condition of mastoid septa, ossicular chain, cortex outside and inside the mastoid bone.

In this clinical patient, the important point was that when mucoperiosteal disease became a bone disorder with enzymatic absorption of the mastoid bone septa and the development of intramastoid empyema, it led to a coalescent mastoiditis condition (Hasso et al. 1989).

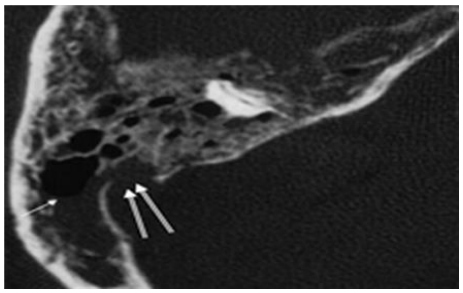

Figure 24. Acute coalescent mastoiditis. Right temporal CT scan axial section showed extensive mastoid debrid with air fluid level (arrow). All septa are thin and irregular. There is also a sigmoid sinus defect (double arrow) which in the reading reveals a suspicion

Subperiosteal abscess

In the additional evaluation of mastoid septa, the internal and external cortex of the mastoid should be examined carefully. A subperiosteal abscess has typically developed from direct extension of the inflammatory debris across the defect in the mastoid cortex.

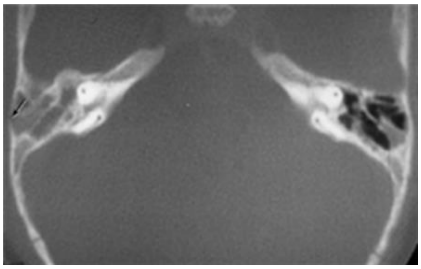

Figure 25. Coalescent otomastoiditis, subperiosteal abscess. Axial axial CT scan temporal showing debris that penetrated the mastoid with the mastoid septa affected when compared to the contralateral

Bezold's abscess

It is analogous to a subperiosteal abscess that occurs when the affected bone is seen at the mastoid end (instead of the external mastoid cortex) on the medial side of the insertion of digastric and sternocleidomastoid muscles. This inflammatory product extends inferiorly along the soft tissue of liver and often forms an abscess.

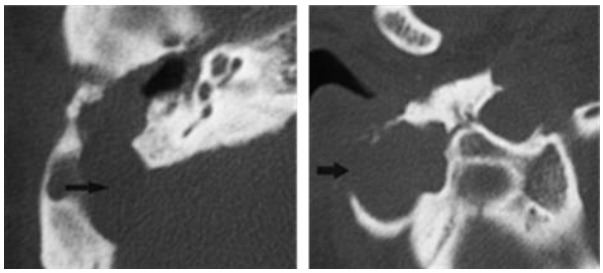

Figure 26. Mastoiditis - Bezold's abscess

Note: (a) Right temporal CT scan axial section showing mastoid debris with a large sigmoid sinus plate defect (arrow). (b) Similar cut that is inferioronly shows information on the tip of the mastoid with a large area of erosion with a large lateral bone defect (arrow).

Chronic otomastoiditis and its complications

Non-healing inflammatory process in the middle ear and mastoid associated with a period of inactivity of an inflammation. Virtually, all patients have a prolonged stage of Eustachian tube dysfunction and decreased intratympanic pressure which is the basis for precipitating factors in various disease processes (Swartz & Harnsberger 1998).

Middle ear effusion

Unexplained middle ear effusions are thought to be associated with invasion of eustachian tube lesions and tensor veli palatini. Trotter's syndrome (unilateral

deafness, pain in the trigeminal nerve and soft tissue of the stiff palate) can form if invasion affects peripheral nerves.

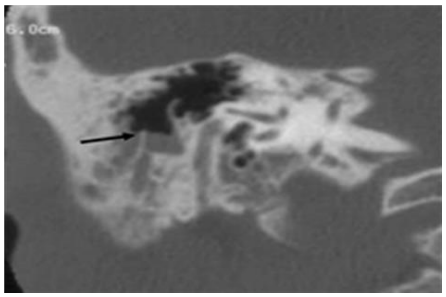

Figure 27. Middle ear effusion. Potential coronal CT scan right temporal showing multiple air fluid levels (arrows)

Inner ear

Labyrinthitis

There are various variations that cause labyrinthitis. The most common symptoms are SNHL (sensorineural hearing loss) and vertigo which may recur. Labyrinthitis has been classified in various ways, one of which is listed in the following table.

Table 2. Classification of labyrinthitis

Deployment Route	Causes	Others	
Tympanogenic	Viral	Serous	Toxic
Meningogenic	Autoimmune	Suppurative	Epidemic
Hematogenic	Bacterial		
Posttraumatic	Luetic		

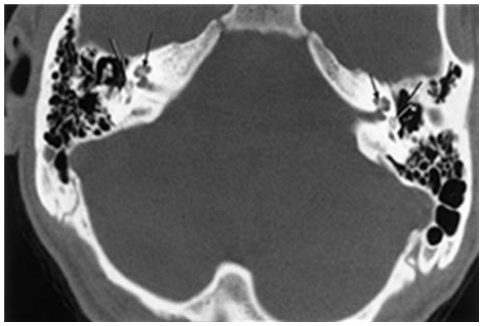

Figure 28. Labyrinth (*meningogenic*) ossification. Temporal axial CT scan section showedclear hardening in the anterior chamber (thick arrow). There was a slight change in ossification at the top of the cochlea (thin arrow)

Neoplasm on CT scan of the temporal bone

Outer ear

Cholesteatoma of the outer ear

Cholesteatoma in the external auditory canal usually occurs without hearing complaints, until the abnormality is enlarged and when it occurs, it will be accompanied by conductive deafness. Advanced cholesteatoma growth can invade mesenchymal tissue with the accumulation of necrotic debris and bone erosion in the form of a bone sequester.

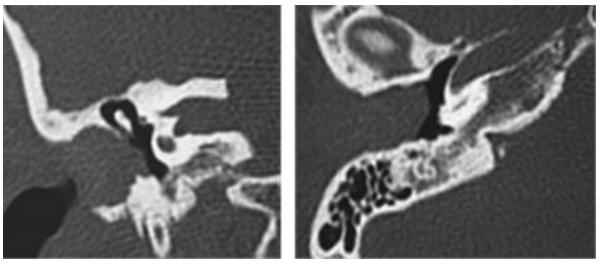

Figure 29. (a) coronal cut CT scan of the right temporal showed a large cholesteatoma filling the external auditory canal and eroding its superior and inferior walls. (b) axial-cut temporal CT scan in the same patient showed expansion of mass and pushes the tympanic tube into the middle ear space

Keratosis obturans

Keratosis obturans can be evaluated best with a CT scan, which shows remodeling or bone changes resulting in diffuse dilation of the external auditory canal.

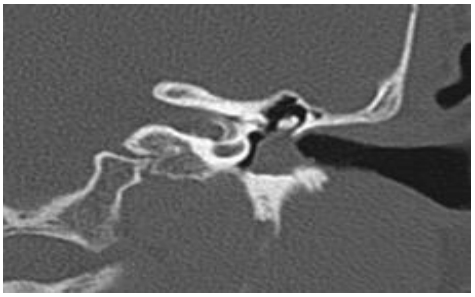

Figure 30. Coronal CT scan of the left temporal showed a soft tissue mass (*keratosis obturans*) in the fundus of external auditory canal and producing several indentations at the base of canal

Polyps

It is said that the incidence of polyps is quite high (52%) with cholesteatoma as an underlying disease, therefore temporal CT scan is useful to determine the extent of the disease and plan of action (Swartz & Harnsberger 1998).

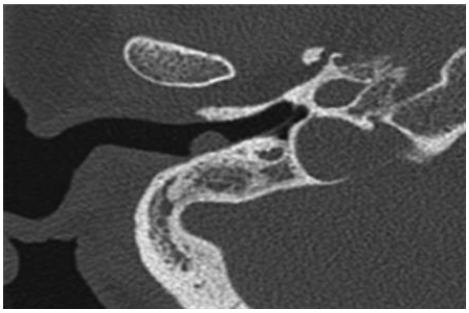

Figure 31. The right temporal axillary CT scan section revealed a small, round polyp in the exposed external auditory canal

Lipoma

Clinically, lipoma is enveloped by epithelium and by physical examination. It is somewhat unclear to diagnose it. The differential diagnosis includes angiomyolipoma. Liposarcoma showed bone erosion on temporal CT scan.

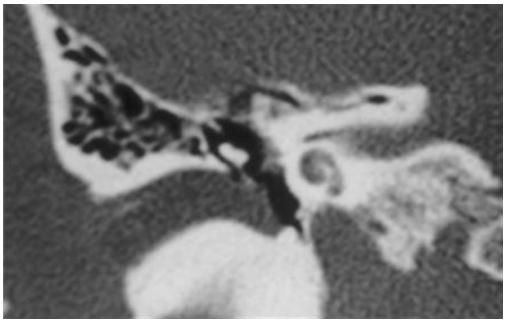

Figure 32. Coronal cut CT scan of the right temporal showed a mass in the external auditory canal, although examination using a bone window setting, the presence of a low density indicated fat direction

Arteriovenous malformations

The outer ear is the most common location for AVMs outside the head and neck. A study concluded about 44 lesions that showed an enlarged development could occur during childhood, adolescence, pregnancy and adulthood (Aspestrand & Kolbenstvedt 1995).

Tumors originating from glands

These tumors grew from cerumen glands or salivary ectopic tissue, usually appeared at age 30 to 60 years and produced otorrhea and hearing loss without pain. On physical examination, a soft tissue mass appeared in the external auditory canal without bone erosion.

Neurofibroma and schwannoma

Schwannomas arising from external auditory canal are very rare (reference). Yet, there are those that involve external auditory canal indirectly, namely schwannomas arising from cranial nerves VII and IX.

Meningioma

Meningiomas most commonly arise from intracranial and spread to the temporal bone and external auditory canal rather than arise from the temporal bone itself. In general, as much as 20% of meningiomas can spread extracranial and occur in the temporal bone. The involvement of the temporal bone in the meningioma can be evaluated with a CT scan.

Exostosis

Most exostoses are found in individuals who have been exposed to cold water frequently. This causes a bone hyperplasia reaction due to exposure to cold water. These lesions consist of a more superficial layer of bone with a denser base layer of bone that has lost vascularization.

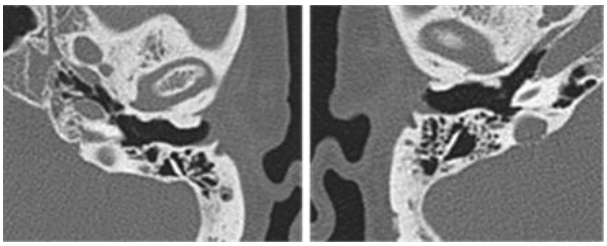

Figure 33. (a) Shaped axial CT scan of the right temporal showing coincidence, exostotic bone, small size in the anterior wall and deeper than the external auditory canal. (b) A left temporal axial CT scan revealed a large bony exostosis on the lateral side of the external auditory canal

Osteoma

CT scan with bone algorithms can help describe in detail the location and plan of surgery.

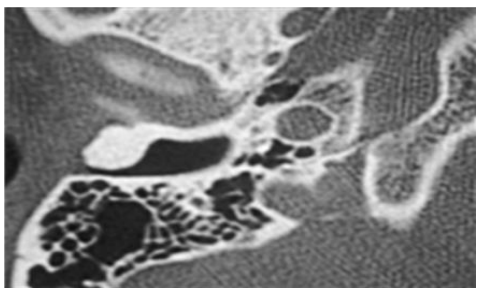

Figure 34. Axial cut CT scan of the right temporal showing a more dense osteoma involving the lateral aspect of the external auditory canal and causing significant narrowing

Carcioma

Squamous cell malignancy has been made in stages called the Pittsburgh Staging Criteria, and has undergone following modifications.

T1 : Only in the external auditory canal, without

any erosion of the bone or soft tissue involved.

T2 : Only in the auditory canal, accompanied by bone erosion (almost the entire thickness of the bone) or soft tissue mass <0.5 cm.

T3 : Erosion of all bone thickness or soft tissue mass <0.5 cm, involving the middle ear or mastoid.

T4 : Involves the cochlea, petrous apex, middle ear wall, carotid canal, jugular foramen, dural, temporomandibular joint, or styloid: facial paresis and soft tissue mass > 0.5 cm.

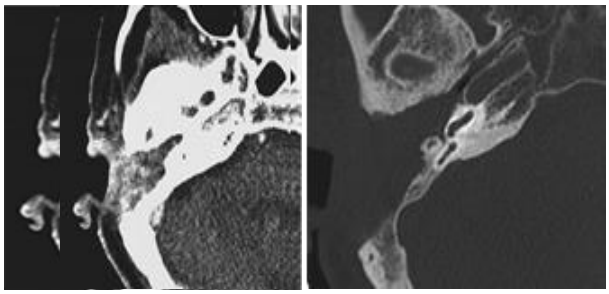

Figure 35. (a) right temporal axial CT scan with soft tissue algorithm showed a non-specific mass. (b) bone algorithm showed that the aggressiveness of mass erodes the mastoid bone and extended to the middle ear.

Tumor extension can also pass through the Santorini fissure to the parotid gland and the TMJ, also across the tympanomastoid suture line to the mastoid bone and through infratemporal fossa to the face.

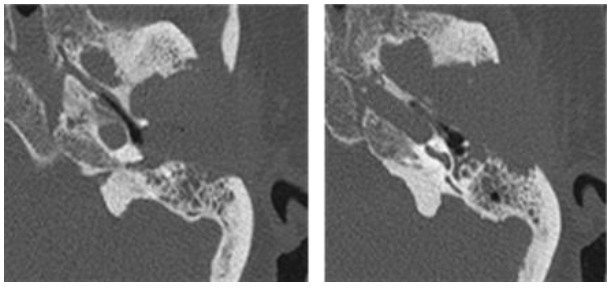

Figure 36. (a) axial cut CT scan of the left temporal showing an adenocarcinoma-like appearance of the left external auditory canal significantly eroding the skull base in the TMJ and mastoid region. (b) axial cut temporal CT scan similar to a lower level shows invasion into the middle ear space and part of the auditory bone is also eroded.

Middle ear

Cholesteatoma of the middle ear and its complications

In fact, cholesteatoma is an inaccurate name, because this lesion is not a neoplasm and can with or without cholesterol crystals, cholesteatoma can be congenital (epidermoid) (Johnson et al. 1983).

Table 3. Middle ear cholesteatoma

	%
Congenital	2
Obtain	98
Pars flaccida, (primer)	82
Pars tensa, (sekunder)	18
Posterosuperior (sinus cholesteatoma)	78
Anterior and inferior	22

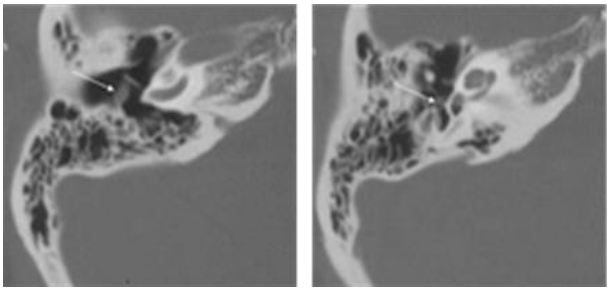

Figure 37. *Cholesteatoma pars flaccida*

Note: (a) axial cut CT scan of right temporal showed Prussak's space soft tissue lesion (arrow). (b) coronal cut CT scan of right temporal showed aural polyp (white arrow) and scutum erosion (black arrow). Coincidentally, it is superior to the malleal ligament (white arrow). Acquired cholesteatoma, pars tensa, (c) axial cut CT scan right temporal showed small soft tissue mass (arrow), and (d) axial cut temporal CT scan with superior cut, confirming mass attached to pyramidal surface.

In the future, if cholesteatoma is not treated properly, there will be various complications associated with bone erosion. Although the pathogenesis of bone damage remains controversial. The factors involved are mechanical, biomechanical and cellular level. Yet, there is a simple concept of a pressure mechanism that causes a direct effect of keratin expansion and is associated with debris. This tendency for bone erosion leads to changes in the middle ear cleft associated with cholesteatoma complications, namely ossicle erosion, labyrinthine fistula, involvement of facial nerve canal, expansion of petrous apex, total deafness and automastoidectomy.

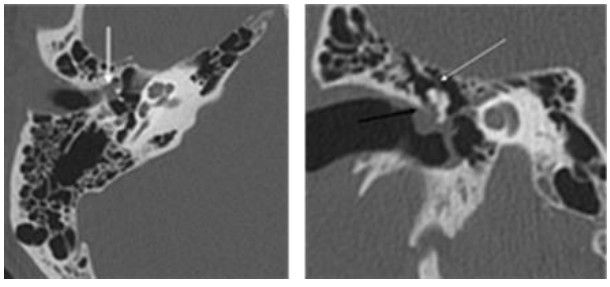

Figure 38. Cholesteatoma obtained in middle ear

Note: (a) axial section CT scan of the right temporal showing a scattered atticoantral mass which has eroded the incus body (white arrow). The lateral cortex of the semicircularis canal is intact (double black arrows), (b) coronal cut CT scan of the right temporal shows changes in the attic due to cholesteatoma (double black arrows), the scutum is still intact (white arrow). Obtained Cholesteatoma, fistula labyrinth (a) axial section (b) coronal section CT scan of the right temporal showing a holotympani mass with erosions in the lateral semicircularis canal cortex (arrow), indicating impending fistulation. This should be reported for the clinician's reference, noting that it is more anterior to the sigmoid sinus (S).

Paraganglioma

Paraganglioma usually grows from the glomus of the body associated with the tympanic branch of the glossopharyngeal nerve (Swartz and Harnsberger, 1998).

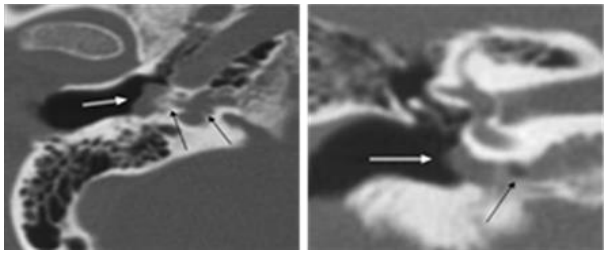

Figure 39. Paraganglioma (*glomus tympanicum*) (a) axial section (b) coronal section CT scan right temporal showing a mass with an even edge along the surface of the promontorium (white arrow),noted sublabyrin infiltration (black arrow)

Congenital cholesteatoma

In general, congenital cholesteatoma has a thinner and flatter matrix when it lies directly on intact tympanic membrane (due to pressure effects). Congenital cholesteatoma is more often unilateral, but bilateral cases have also been reported.

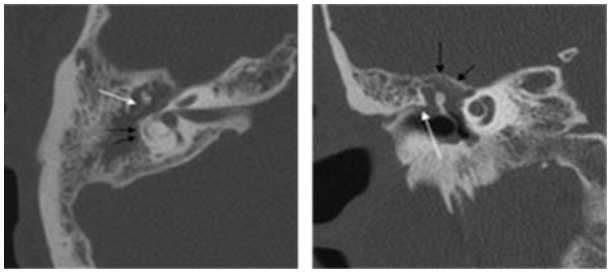

Figure 40. *Congenital cholesteatoma* (a) axial section (b) coronal section CT scan of left temporal showed soft tissue mass anterior to the tympanic space (arrow) recorded normal incudostapedial articulation (white arrow in image a) and lateral malleal ligament (white arrow in image b)

Schwannoma

There is a lack of CT compared to MRI which can differ between paraganglioma and schwannoma. It is important to know that schwannoma in the middle ear occurs more often without facial nerve palsy. The schwannoma is usually well coiled and arises from the main trunk of the facial nerve.

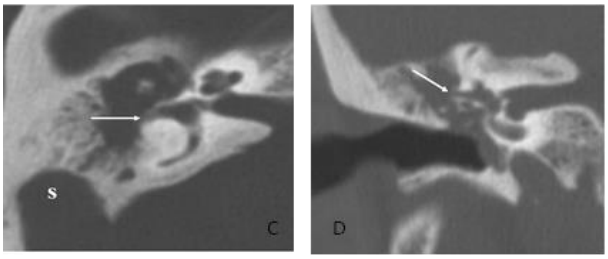

Figure 41. Middle ear schwannoma

Rhabdomyosarcoma

In this case, CT examination helps to demonstrate the extent of destruction of bone with the use of contrast. It will help to predict more associated forms of cholesteatoma (Swartz & Harnsberger 1998).

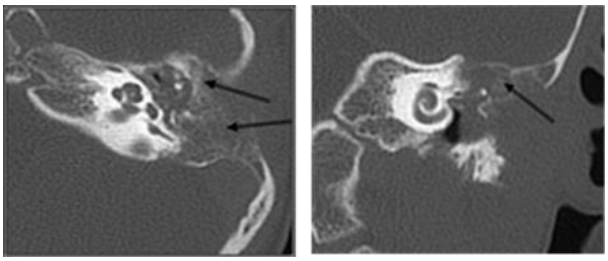

Figure 42. *Rhabdomyosarcoma*

Note: (a) Axial cut, (b) coronal cut left temporal CT scan showed middle ear mass with permeative type bone destruction.

Giant cell tumor

GCT is a very rare lesion of the temporal bone, which can extend to the middle ear and infratemporal fossa and tends to be quite large (Swartz & Harnsberger 1998).

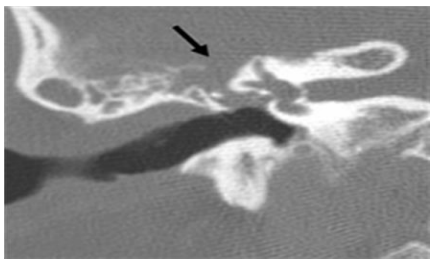

Figure 43. Giant cell tumor coronal cut right temporal CT scan. Soft tissue lesions appear in the attic and tegmental air cells with the distribution of damage to tympani tegmen (arrow)

Inner ear

Intralabyrin schwannoma

Intralabyrin schwannoma is rare, can occur occasionally and is more frequent in patients with NF-2, but most cases are more sporadic. There is a drawback to examining a CT scan rather than an MRI to look for structural abnormalities in the soft tissue.

Table 4. Intralabyrin schwannoma origin grouping

Based on location on the labyrin	IAC involvement	Middle ear involvement
Intracochlear Intravestibular Intravestibulococh-lear	Transmodiolar (cochlea and IAC) Transmacular (vestibule and IAC)	Transotic (Middle ear, labyrinth, and IAC) Tympanic Labyrinthine (Middle ear and labyrinth)

Source: Kennedy RJ et al (2004)

Fibrous dysplasia

Pagetoid, sclerotic and cystic are depictable forms of fibrous dysplasia and these disorders are adequately described by temporal CT scan.

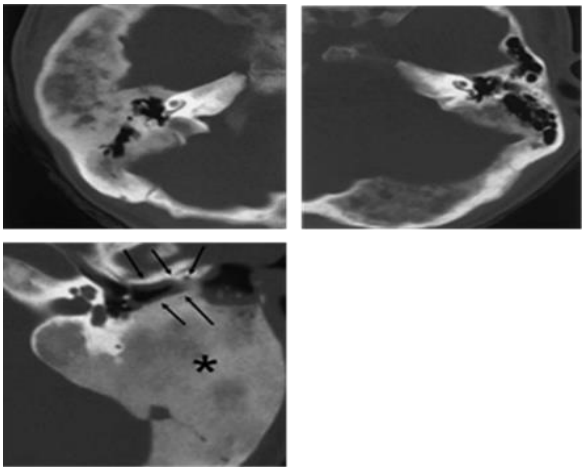

Figure 47. Fibrous dysplasia, pagetoid (a) axial cut CT scan right temporal, (b) axial CT scan left temporal, showed widespread heterogeneous thickening of the squamous and petrous temporal bone, (c) fibrous dysplasia, sclerotic. Extensive sclerotic appearance of the mastoid is characterized by increased bone volume (*). Changes in occlusion of external auditory canal were noted, often with arrow-pointed abnormalities

Strength and limitation

HRCT offers high resolution images of the temporal bone, providing detailed information on the structure and soft tissue components. Soft tissue evaluation is easier to analyze compared to MRI.HRCT protocol for temporal bone allows for easier analysis and identification of standard structures, congenital abnormalities, and pathological problems. HRCT can expose patients to radiation, which can be harmful in high doses. Tilted image reconstruction can introduce artifacts that may affect the accuracy of the images.HRCT may not be suitable for patients with certain medical conditions, such as pregnancy or kidney problems, due to the use of contrast agents.HRCT is not suitable for assessing some soft tissue structures, such as the meninges or blood vessels, which may require MRI or other imaging modalities.

CONCLUSION

There were so many and detailed potential abnormalities that occurred in the temporal bone. Its constituent structures had made a prospective radiologist forced to be more observant and thorough in evaluating the area, with the guidance and sample images obtained. It was expected that it could help prospective radiologists to described structural abnormalities.

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Conflict of interest

None.

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Author contribution

WF and IBGR were conseptual idea, study design, collected data. BGR was write and revised the manuscript. WF was analysis data, checking grammar, and validation the manuscript to publish.

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On the other hand, **Invited Literature Review** provides a detailed and comprehensive narrative analysis of recent developments in a specific topic in medicine and highlights important points that have been previously published. The text consists of Abstract, Introduction, highlights, any subheadings as needed by the author(s), Strength and limitations, Conclusion, Acknowledgement, Conflict of Interest, Funding Disclosure, Author Contribution, and References. The text is relatively long compared to other paper categories, typically up to 15 manuscript pages or 4,000 words with approximately 30-50 reference list to comprehensively cover all the major published work.

Meta-Analysis

Meta-analysis is a statistical analysis combining the results of multiple scientific studies, analyzing multiple scientific studies addressing the same question, with each individual study reporting measurements that are expected to have some degree of error. The total number of tables and figures are limited, advisably not more than five, and references are minimally 20 from the last 10 years before the date of submission. The text consists of Abstract, Highlights, Introduction, Materials and Methods, Results, Discussion, Strength and limitations, Conclusion, Acknowledgement, Conflict of Interest, Funding Disclosure, Author Contribution, and References.

References

References are advisably not to exceed 35 in number but not less than 20, and should in general be limited to the last ten (10) years from the submission date. Avoid using abstracts as references. Information from manuscripts submitted but not yet accepted should be cited in the text as “unpublished observations” with written permission from the source. Papers accepted

but not yet published may be included as references; designate the journal and add "Forthcoming". Avoid citing "personal communication" unless it provides essential information not available publicly, name the person and date of communication, and obtain written permission and confirmation of accuracy from the source of personal communication. Authors are recommended to use reference management software, in writing the citations and references such as Mendeley®, Zotero®, EndNote®, and Reference Manager®.

Here are some examples of the references:

1. Journal

Up to three authors, list all the authors.

Halpern SD, Ubel PA, Caplan AL (2002). Solid-organ transplantation in HIV-infected patients. N Engl J Med 347, 284-287 (doi: …….)

More than three authors, list the first three authors, followed by et al.

Rose ME, Huerbin MB, Melick J, et al (2002). Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res 935, 40-46 (doi: …….)

2. Book

Two to three authors, list all the authors.

Sambrook J, Russerl DW (2001). Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, New York.

More than three authors, list the first three authors, followed by et al.

Reece JB, Lisa AU, Peter VM, et al (2010). Campbell biology. Pearson, London.

Chapter in a book

Meltzer PS, Kallioniemi A, Trent JM (2002). Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW (eds). The genetic basis of human cancer, New York, McGraw-Hill, p 93-113.

Electronic book/ E-book

Chapter from an electronic book

Darwin C. On the origin of species by means of natural selection or the preservation of favoured aces in the struggle for life [Internet]. London: John Murray; 1859. Chapter 5, Laws of variation. [cited 2010 Apr 22]. Available from: http://www.talkorigins. org/faqs/origin/ chapter5.html.

Full-text electronic book

Macdonald S. editor. Maye's midwifery 14th ed. [eBook]. Edinburgh: Bailliere Tindall; 2011 [cited 2012 Aug 26]. Available from: Ebrary.

3. Proceeding

Offline proceeding

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

Online proceeding

Muller S, editor. Proceedings of the 10th international conference on head-driven phrase structure grammar [Internet]; 2003 Jul 18-20; East Lansing (MI). Stanford (CA): CSLI Publications; 2003 [cited 2017 Nov 16]. Available from: http://web.stanford.edu/group/ cslipublicationsSta/cslipublications/HPSG/2003/toc. shtml.

4. Theses/ Dissertation

Offline theses/dissertation

Kay JG. Intracellular cytokine trafficking and phagocytosis in macrophages [dissertation]. St Lucia, Qld: University of Queensland; 2007

Online theses/dissertation

Pahl KM. Preventing anxiety and promoting social and emotional strength in early childhood: an investigation of risk factors [dissertation on the Internet]. St Lucia, Qld: University of Queensland; 2009 [cited 2017 Nov 22]. Available from: https://espace.library.uq.edu.au/view/UQ:178027.

5. Homepage/ Website

Cancer-Pain.org (2002). New York: Association of Cancer Online Resources, Inc.; c2000-01. [updated 2002 May 16]. Available from http://www.cancer-pain.org/. Accessed July 9, 2002.


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