ANALYSIS OF MICRONUTRIENT INTAKE, MATERNAL WEIGHT GAIN DURING PREGNANCY, AND PASSIVE SMOKER ON NON-SYNDROMIC OROFACIAL CLEFT INCIDENCE AT HARAPAN KITA WOMEN AND CHILDREN HOSPITAL

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ABSTRACT

A congenital facial anomaly that manifests as a cleft lip, cleft palate, or both—without the involvement of other organ abnormalities—is known as a non-syndromic orofacial cleft (OFC) (Tobing, 2017). According to the Indonesian Ministry of Health (2019), OFC represents 0.2% of disabilities among Indonesian children aged 24 to 59 months. The causes of OFC are multifactorial. In Indonesia, approximately 7,500 new OFC cases are reported annually, with a rising trend (Purwitasari et al., 2020). This study aimed to investigate the association between micronutrient intake, maternal weight gain during pregnancy, and maternal exposure to secondhand smoke with the incidence of non-syndromic OFC. A case-control study was conducted at Harapan Kita Women and Children Hospital in Jakarta, involving 35 participants in both the case and control groups. Data were collected from both primary and secondary sources. Primary data were obtained through direct interviews with respondents, while secondary data were sourced from maternal and child health (MCH) books. Micronutrient intake was measured using a semi-quantitative food frequency questionnaire (SQ-FFQ). Data analysis was performed using Microsoft Excel 365 and SPSS version 25.0 for Windows, applying logistic regression. The findings indicated that low folic acid and zinc intake, insufficient maternal weight gain during pregnancy, and maternal exposure to secondhand smoke were significantly associated with the development of non-syndromic orofacial cleft ($p < 0.05$).

Keywords: *Orofacial Cleft, Micronutritent Intake, Weight Gain, Passive Smoker*

INTRODUCTION

Orofacial cleft (OFC) is a birth defect characterized by a gap in the palate (cleft palate), the lip (cleft lip), or both (cleft lip and palate). In Indonesia, the number of OFC cases has been rising steadily, with an average increase of 7,500 new cases annually and the trend continuing to grow (Purwitasari et al., 2020).

The development of facial structures is a synchronized process involving the formation of the lips, palate, nasal passages, and oral cavity, which takes place between the 4th and 12th weeks of gestation (Nasreddine et al., 2021). Nutritional intake in pregnant women has increased to meet the increasing nutritional needs during pregnancy. Pregnant women who experience nutritional deficiencies will experience deficiencies in nutrients needed for fetal growth and development, such as folic acid, vitamin B12, and zinc (Dien et al., 2018).

A crucial nutrients for the synthesis of DNA and the metabolism of amino acids is folic acid. During pregnancy, the need for folic acid rises to support the fetus's growth and ensure an adequate blood supply from the mother (Haryadi et al., 2022). The gene involved in non-syndromic OFC events is MTHFR (methylenetetrahydrofolate reductase). As a cofactor in a number of metabolic events, including the conversion of the amino acid homocysteine to methionine, which results in methylation, MTHFR will change 5.10-MTHF into 5 MTHF, the active metabolite of folic acid. Homocysteine buildup and a decrease in the active form of folic acid will hinder tissue growth

 Received 22-08-2024, Accepted 31-12-2024, Published online 31-05-2025.

and cell differentiation during embryogenesis. (Inchingolo et al., 2022)but their etiology has yet to be clarified. OFCs affect different structures and functions with social, psychological and economic implications in children and their families. Identifying modifiable risk factors is mandatory to prevent the occurrence of non-syndromic OFCs (NSOFCs. This will lead to the occurrence of OFC.

Vitamin B12 is one of the important vitamins for pregnant women. Vitamin B12 together with folic acid plays a role in preventing the incidence of OFC (Bailey et al., 2015). Vitamin B12 deficiency conditions cause folate to be trapped in an unusable methyl form (Munger et al., 2021). If folate cannot be used, it will have an impact on genomic disorders that lead to malformations (Green et al., 2017).

Zinc is an essential mineral that has benefits for physiological functions, growth, reproduction, and the immune system (Jarosz et al., 2017). According to research by Abdulla et al. (2016), the control group's average homocysteine (Hcy) concentration was 9.5 µmol/L, whereas the CL±P non-syndromic case group›s Hcy concentration increased to an average of 18.4 µmol/L. An increase in Hcy will cause a disruption in the fusion of the palate which can lead to cleft palate.

Maternal weight gain during pregnancy is a determinant of the final health outcome of both the mother and the newborn baby. The weight gain of pregnant women is determined by the nutritional status of the mother before pregnancy. The weight gain of pregnant women who are underweight or overweight will have various adverse effects on the baby who is born, such as premature birth, the birth of a baby with a low birth weight, and congenital abnormalities in the baby who is born (Simko et al., 2019).

Maternal weight gain that does not exceed recommendations is related to the fulfillment of nutrients needed during pregnancy. If the mother experiences insufficient weight gain during pregnancy, she is at risk of folic acid deficiency and other important nutrients during pregnancy. In addition, inappropriate maternal weight gain can also interfere with fetal growth and development, including the development of facial structures. Meanwhile, if the mother experiences excessive weight gain during pregnancy, there is a risk of nutritional imbalance. Obese mothers may have a diet high in calories but low in nutritional quality, which can lead to micronutrient deficiencies essential for fetal development.

The likelihood of developing cleft lip (CL) and cleft palate (CP) is increased by 1.4 and 2.1 times, respectively, among passive smokers (Sato et al., 2021).

OFC can reduce the quality of life, health, and socioeconomic well-being of families or individuals. The limited research on OFC associated with nutrition and the environment has led to a lack of awareness about preventing OFC increases. Therefore, it is important to understand the risk factors and provide awareness on how to prevent exposure to these factors.

METHODS

This research employed a case-control study design. Data collection was carried out through interviews with mothers of infants diagnosed with orofacial clefts (OFC) and mothers of healthy infants at Harapan Kita Women and Children Hospital in Jakarta. The study took place between December 2023 and March 2024. Ethical approval was granted by the Ethics Committee of Bogor Agricultural University (approval number 1148/IT3.KEPMSM-IPB/SK/2024) and the Ethics Committee of Harapan Kita Children's Hospital Jakarta (approval number IRB/15/02/ETIK/2024).

The number of samples obtained based on the formula for calculating the number of samples for case control. According to Angulo-Castro et al. (2017), with a minimum OR of 3.27, a power of 90%, an alpha level of 5%, and the addition of 10%, the minimum sample size required was 35 individuals per group or a total of 70 participants. Data were obtained through direct interviews with respondents. The selection of subjects was conducted using convenience sampling, taking into account specific inclusion and exclusion criteria.

The inclusion criteria for the case group were: 1) mothers with infants under one year old diagnosed with non-syndromic orofacial cleft; 2) possession of a Maternal and Child Health Book (KIA); and 3) receipt of regular or occasional OFC treatment at Harapan Kita Women and Children

Hospital. For the control group, the inclusion criteria included: 1) mothers with infants under one year old or who had recently given birth to babies without non-syndromic OFC; 2) possession of a KIA book; and 3) who had received care or delivered at Harapan Kita Women and Children Hospital.

Maternal age was grouped into three categories: < 20 years, 20 - 35 years, and > 35 years, based on the Ministry of Health (2016) which classifies pregnancy at < 20 years or > 35 years as a high-risk pregnancy. However, in this study, age was divided into two groups: a non-risk group for mothers who became pregnant at 20 - 35 years old, and a risk group for mothers who became pregnant at < 20 years old or > 35 years old.

Mother's education level was grouped into two categories according to the researcher's assumption: high if the mother had a diploma/ graduate degree and low if the mother did not attend school, elementary school, junior high school, and senior high school. Meanwhile, the mother's occupation was grouped into two categories: at risk of causing OFC and not at risk of causing OFC.

Maternal occupations before and during pregnancy in this study were grouped into two categories, namely risky and non-risky based on the researcher's assumptions. Maternal occupations that are at risk of causing OFC are occupations that get exposure to odors from chemicals, radiation, pesticides, or other compounds harmful to pregnancy. The data were obtained through the interviews with mother.

The nutrition intake data were obtained using the semi-quantitative food frequency questionnaire (SQ-FFQ). The data of mothers food intake were taken by interviewing their eating habits during the first five weeks of pregnancy. The data were analyzed by using the Nutrisurvey 2007 application. The results of nutritional intake (folic acid, vitamin B12, and zinc) were categorized into two groups, normal if ≥ 77% RDA and deficit if < 77% RDA (Gibson, 2005).

The nutritional status of pregnant women based on weight gain during pregnancy is classified into four categories according to the IOM (2009) guidelines: underweight (12.5–18 kg), normal weight (11.5–16 kg), overweight (7–11.5 kg), and obese (5–9 kg). In this study, these categories were further simplified into two groups: appropriate and inappropriate maternal weight gainThe category is appropriate if the weight gain is in accordance with the IOM guidelines and the category is inappropriate if the weight gain is less than the IOM guidelines or more than the IOM guidelines. Measurement of weight gain during pregnancy based on the mother's weight before pregnancy and the mother's weight during the third trimester. The data were obtained through the MCH book and interviews with mother. The total maternal weight gain during pregnancy was evaluated against the IOM guidelines, which are tailored to the mother's nutritional status prior to pregnancy.

Indicators of maternal nutritional status before pregnancy using body mass index (BMI). BMI categories based on (WHO, 2000) are grouped into five categories namely thin (<18.5 kg/m2), normal (18.5 - 22.9 kg/m2), overweight (23.0 - 24.9 kg/m2), obese 1 (25 - 29.9 kg/m2), obese 2 (> 30 kg/m2). In this study, the nutritional status of mothers before pregnancy was grouped into two groups, namely not obese and obese. The not obese category is mothers who BMI within the status of neither obesity 1 nor obesity 2 according to WHO (2000). While the obese category if the mother's BMI is in the category of obesity 1 and obesity 2 based on WHO (2000). The data were obtained through the MCH book and interviews with mothers.

Passive smoking mothers are mothers who have husbands or family members at home who smoke or mothers who are in a smoking work environment. In this study, it was grouped into two categories, namely yes and no. The category yes if the mother was exposed to cigarette smoke during pregnancy. No category if the mother was not exposed to cigarette smoke during pregnancy.

Data were analyzed using a logistic regression test to determine the relationship and strength of the relationship.

RESULTS AND DISCUSSIONS

Characteristics of the Infant and Mother

The participants were 70 mothers, consisting of 35 mothers who had infants with OFC and 35

https://doi.org/10.20473/mgi.v20i2.202–210

Table 1. Characteristics of Mothers

Variable	n (%)	
	Case	Control
Mother's Age at Pregnancy		
Not a risk	30 (85.7)	28 (80)
At risk	5 (14.3)	7 (20)
Mother's Education Level		
High	21 (60)	29 (82.9)
Low	14 (40)	6 (17.1)
Mother's Occupation Before Pregnancy		
Non-risky	29 (82.9)	34 (97.1)
Risky	6 (17.1)	1 (2.9)
Mother's Occupation During Pregnancy		
Non-risky	31 (88.6)	34 (97.1)
Risky	4 (11.4)	1 (2.9)

mothers who had normal infants. In this study, the majority of infants from both the case and control groups were male, accounting for 51.4%. Among the types of clefts observed, the most common was a combination of cleft lip and palate at 80%, followed by cleft lip alone at 11.4%, and cleft palate alone at 8.6%. The maternal characteristics related to this study are presented in Table 1.

Based on Table 1, maternal age at the time of pregnancy in both case and control groups was in the non-risk category (20–35 years). In the case group, there were 14.3% of mothers who were in the risky gestational age category (< 20 years and > 35 years), while in the control group, there were 20% of mothers who were in the risky gestational age category. The average age of mothers during pregnancy in the case group was 29.7 ± 5.2, and in the control group it was 31.3 ± 5.0.

Most of the mothers in this study in both the case and control groups took more education up to college. However, in the case group, there were 40% of mothers who had low education, while in the control group, there were 24.3% of mothers who had low education. The distribution of maternal occupations before and during pregnancy showed that the average mother in both case and control groups worked in occupations that did not have the potential to cause OFC risk. However, in the case group, there were 17.1% of mothers who worked before pregnancy in occupations that could potentially pose a risk to OFC.

Occupations that have the risk of OFC are jobs that are exposed to many chemicals, pesticides, or heavy metals. Mothers who work in environments with exposure to chemicals, pesticides, heavy metals, or other toxic substances (such as in manufacturing industries, agriculture, or chemical laboratories) have a higher risk of giving birth to a child with an orofacial cleft (Spinder et al., 2017). Exposure to such materials during the first trimester, when the fetus' facial organs are forming, is critical. The types of work of mothers in the case group that potentially pose a risk of OFC include architects, printers, dentists, pharmacists, and working in salons. Meanwhile, in the control group there were 2.9% of mothers whose occupations were at risk of causing OFC, with the type of work being working in a beauty clinic.

Relationship between Independent variables and OFC incidence

The independent variables analyzed in this study were micronutrient intake (folic acid, vitamin B12, and zinc), maternal weight gain during pregnancy, and passive smoking. The relationship between the independent variables and the incidence of OFC can be seen in Table 2.

Based on Table 2, folic acid intake from foods consumed by mothers in this study were significantly associated with orofacial cleft (p = 0.001). Deficient folic acid intake during the first five weeks of pregnancy increased the incidence of OFC by 4.9 times (OR = 4.9; 95% CI: 1.8-13.6). The results of this study are in line with research conducted by Mendonca (2020) on respondents in hospitals in Bangalore, India, which found that folic acid sourced from food was associated with the incidence of OFC (p = 0.005).

Folate is part of the water-soluble B vitamins. Sources of folate come from legumes and vegetables (Bendahan et al., 2020). The required folic acid in women aged 19-49 years is 400 mcg a day and will increase during pregnancy trimester one to three by 200 mcg a day. So the total folic acid needed by pregnant women is 600 mcg a day.

Folic acid deficiency during pregnancy can cause congenital birth defects (Purwitasari et al., 2020) especially during organ formation due to

Table 2. Relation of Independent Variables and OFC Incidence

Variable	n (%)		*p value*	OR (95% CI)
	Case	Control		
Folic Acid Intake				
Normal	8 (22.9)	22 (62.9)		1
Deficit	27 (77.1)	13 (37.1)	0.001*	5.7 (2.0-16.2)
Vitamin B12 Intake				
Normal	27 (77.1)	28 (80)		1
Deficit	8 (22.9)	7 (20)	0.771	1.2 (0.3-16.2)
Zinc Intake				
Normal	9 (25.7)	22 (62.9)		1
Deficit	26 (74.3)	13 (37.1)	0.002*	4.9 (1.8-13.6)
Mother's BMI Status				
Not obese	25 (71.4)	24 (68.6)		1
Obese	10 (28.6)	11 (31.4)	0.794	0.9 (0.3-2.4)
Maternal Weight Gain During Pregnancy				
Appropriate	23 (65.7)	31 (88.6)		1
Inappropriate	12 (34.3)	4 (11.4)	0.029*	4.0 (1.2-14.2)
Mothers as Passive Smoker				
No	7 (20)	26 (74.3)		1
Yes	28 (80)	9 (25.7)	0.000*	11.6 (2.8-35.5)

the role of folic acid, which is the formation of the nervous system, bone formation, hormone regulation, and the center of intelligence (Manuaba IBG, 2012).

Methylenetetrahydrofolate reductase (MTHFR) plays a key role in the metabolism of folic acid and homocysteine (Hcy). It relies on folic acid for DNA synthesis and the processing of vitamin B12, which is essential for converting homocysteine into methionine (Nasroen et al., 2022). Hcy is formed during methionine metabolism in cells. Methionine is useful for forming proteins derived from food. MTHFR is an important enzyme in folate metabolism that converts 5,10-*methylene*-THF menjadi 5-*methyl*-THF (Garland et al., 2020). This form serves as the primary circulating folate and the main source of methyl groups for the remethylation of homocysteine (Hcy) into methionine (Nasroen et al., 2022). If folate intake is low, it will lead to an increase in Hcy levels, which is dangerous for pregnant women. The impact will be in the form of abortion, placental infarction, and fetal growth disorders.

Furthermore, elevated homocysteine (Hcy) levels can impair the function of the MTHFR enzyme. Reduced MTHFR activity leads to lower folate levels and higher concentrations of Hcy in the bloodstream. Consequently, the synthesis of methionine is disrupted, which negatively impacts DNA methylation, gene expression, and developmental processes, including orofacial development(Gamayani et al., 2015).

Zinc intake in this study has a significant relationship with orofacial cleft (p = 0.002). Inadequate zinc intake during the first five weeks of pregnancy was associated with a 4.9-fold increase in the risk of developing OFC (OR = 4.9; 95% CI: 1.8-13.6). A study carried out by Jara-Palacios et al. (2018) found that mothers who had infants with non-syndromic OFC had lower plasma zinc levels when compared to healthy mothers. This is supported by a study carried out by Ni et al. (2019) who found that cord tissue of infants who did not have OFC had higher zinc levels compared to the case group in the study.

Zinc is a vital mineral that plays an important role in physiological functions, growth, reproduction, and immune system support (Jarosz et al., 2017). Zinc required for women aged 19 - 49 years is 8 mg a day and will increase when women experience the first trimester of pregnancy which is an addition of 2 mg and in the second trimester and third trimester of 4 mg a day. Zinc deficiency can adversely affect the mother and fetus and subsequent birth (Hamid et al., 2023). Zinc can

be obtained through the consumption of animal products such as lamb, liver, beef, chicken, oysters and lobster (Uwitonze et al., 2020). Vegetables, nuts and seeds also contain zinc.

The risk of non-syndromic CL/P is influenced by single nucleotide polymorphism (SNP) patterns (Abdulla et al*.,* 2016). During embryonic development, zinc finger proteins are crucial for gene regulation. Zinc is involved in the zinc-dependent enzyme methionine synthase's (MS) conversion of 5-methyl tetrahydrofolate to tetrahydrofolate. The enzymes MTHFR and MS cooperate to control the levels of homocysteine (Hcy). One kind of amino acid in the body that might lead to health issues if it is present in excess is homocysteine. Women during the initial five weeks or the first trimester of pregnancy have a recommended portion of food according to the Balanced Nutrition Guidelines. Folic acid, vitamin B12, and zinc intake can be obtained in animal, vegetable, vegetable, and fruit side dishes. The recommended intake for pregnant women in the first trimester includes 200 grams of animal-based side dishes, 200 grams of plant-based side dishes, 400 grams of vegetables, and 400 grams of fruit.

In this study, pregnant women in the control group consumed a higher average amount of food during the first five weeks of pregnancy compared to those in the case group. The only exception was in the vegetable food group, where the control group had a lower average intake (267 grams) than the case group (348 grams). Despite this, neither group met the recommended vegetable intake. Additionally, mothers in the case group did not meet the recommended portions for vegetable side dishes, while those in the control group did. Conversely, both groups exceeded the recommended intake for fruit consumption.

Through the amniotic fluid, homocysteine will enter the fetus and cause the palatal mesenchyme to undergo apoptosis, preventing palate fusion. According to a study by Abdulla et al. (2016), the control group's average Hcy concentration was 9.5 µmol/L, whereas the CL±P non-syndromic case group's average Hcy concentration increased to 18.4 µmol/L.Inappropriate weight gain during pregnancy can lead to increased adverse risks for both mother and child. One of the risks that can be caused by non-optimal weight gain is the occurrence of congenital abnormalities in babies who are born.

Maternal weight gain that was not inappropriate with IOM guidelines had a significant association with the incidence of OFC (p=0.029). This study is supported by findings from Choi et al. (2022) which showed that mothers who experienced weight gain that was not in accordance with recommendations during pregnancy were at higher risk of congenital abnormalities in the babies born. Therefore, mothers need to pay attention to weight gain during pregnancy so that it is in accordance with recommendations.

Underweight gain during pregnancy is associated with poor food intake during pregnancy or excessive nausea and vomiting during pregnancy. These conditions lead to low fulfillment of nutrients required for fetal growth and development such as folic acid, zinc, and vitamin B12. This causes the fetus to experience failure in fetal development which can cause abnormalities in the baby to be born, one of which is OFC.

Exposure to cigarette smoke is very difficult for mothers to avoid. Mothers can be exposed to cigarette smoke both from family members and from the environment where they work. Often, mothers are less aware of the dangers that can be caused by exposure to cigarette smoke. Numerous dangerous compounds are present in cigarette smoke that is emitted into the atmosphere when tobacco is not completely burned.Test results of mothers as passive smokers in this study showed a significant relationship with orofacial cleft (p = 0.000). Research by Sakran et al. (2022) also supports the results of this study, which states that passive smoking mothers have an association with OFC (p = <0.001). This study also aligns with the findings of research conducted by Tajrin et al. (2024) which explained that passive smoking mothers are associated with the incidence of orofacial cleft in children in the western and eastern regions of Indonesia.

Mothers as passive smokers have 11.6 times the risk of giving birth to a child with OFC compared to mothers who do not smoke (p-value 0.000, OR 11.6, 95% CI: 2.6–35.5). Maternal exposure to cigarette smoke more than six times

per week may increase the risk of a higher OFC (Pi et al., 2018)2.1. According to the WHO (2013), cigarette smoke inhaled by the mother during pregnancy can harm the development of the unborn child. The combined effects of exposure to toxins contained in cigarettes, hypoxia, and ischemia cause craniofacial developmental disorders (Lopes et al., 2022).

Nicotine inhaled through cigarette smoke can cause narrowing of blood vessels (vasoconstriction), causing decreased blood flow to the placenta (de Andrade et al., 2023). Hypoxia in the fetus is caused by injury to the endothelium as a result of inhaled tobacco, causing rupture of placental blood vessels. This condition causes a decrease in blood supply to the fetus. The bond between carbon monoxide and hemoglobin causes a decrease in oxygen availability to the placenta (Lopes et al., 2022).

CONCLUSION

Deficient intake of folic acid and zinc during the first five weeks of pregnancy, deficient weight gain during pregnancy, and maternal passive smoking are associated with OFC incidence.

To prevent deficiencies in folic acid and zinc intake, mothers should eat a diverse and nutritionally balanced diet starting when planning for pregnancy. They should consume foods such as green vegetables, a variety of fruits, meat, nuts and seeds (beans, peas, almonds, kidney beans), and dairy products to fulfill the source of folic acid and zinc. They should avoid foods that can inhibit the absorption of folic acid and zinc such as excessive tea or coffee. If there is a decrease in appetite during pregnancy, folic acid and zinc can be supplemented.

Regarding maternal nutritional status based on weight gain during pregnancy, mothers can first record their pre-pregnancy weight and then track their weight gain through regular check-ups at hospitals or health centers.

One of strategies to reduce maternal exposure to cigarette smoke is to provide information about the dangers of cigarette smoke for pregnant women and fetuses through mass media, seminars, or counseling at health centers. The government can cooperate with companies or neighborhoods to implement smoke-free zones in the workplace or public areas and can provide special spaces for smokers.

A key strength of this study lies in its focus on the relatively limited discussion of nutritional risk factors for non-syndromic orofacial cleft in Indonesia. As such, the findings can offer valuable nutritional recommendations for preventing the occurrence of non-syndromic OFC. Meanwhile, the weakness of this study is that the sample used is small so that it is less representative for the wider population. In addition, there is a risk of recall bias when collecting nutrient intake data using SQ-FFQ because respondents must remember the food consumed in the first five weeks of pregnancy.

ACKNOWLEDGMENT (if necessary)

The authors are grateful to Research Collaboration Indonesia (RKI) for supporting and funding this research. In addition, Harapan Kita Women and Children Hospital for giving permission to conduct research at the hospital.

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