Available online: https://e-journal.unair.ac.id/jps

Original Research

Identification of Potential Diagnostic Markers for Depressive Disorders using Urinary Biomarkers of N-Methylnicotinamide and Hippuric Acid

Nadila Apriola Susanto[1], Uswatun Hasanah[2], AL-Bidarri Tsamira Annafila Sutrisno[2], Handini Risma Hani[2], Sekar Asih[2], Sulistyo Mulyo Agustini[3]

[1]Faculty of Psychology, University of Muhammadiyah Malang, Indonesia
[2]Faculty of Medicine, University of Muhammadiyah Malang, Indonesia
[3]Departement of Clinical Pathology, Universitas Muhammadiyah Malang, Indonesia

Received: February 5, 2024
Accepted : July 8, 2024
Published Online : November 1, 2024

Correspondence Author:
Email: nadilaapriola.s@gmail.com

Abstracts

Introduction: The diagnostic methodology for depressive disorders, relying on symptom clusters, has inherent limitations in ensuring heterogeneity levels. Consequently, this presents a notable risk of inevitable diagnostic errors in mental health assessments. Therefore, advocating for objective diagnostic approaches through empirical testing in clinical settings becomes crucial for individuals dealing with depressive disorders. This study aims to identify the effectiveness of urine as a diagnostic support for depressive disorders using the N-Methylnicotinamide and Hippuric Acid biomarkers. **Methods:** This study used 13 urine samples from patients with depressive disorders and 13 normal urine samples. It used ELISA methods with observational analytic and cross-sectional designs. **Results:** The results showed that the N-methylnicotinamide biomarker had a relationship with depressive disorders with a correlation value of 0.867, while hippuric acid obtained a correlation value of 0.692. Besides, the N-Methylnicotinamide and Hippuric Acid biomarkers showed differences in the urine of depressive disorder and normal patients with significance values of 0.000 and 0.001 for the N-Methylnicotinamide and Hippuric Acid biomarkers, respectively. In addition, the Relative Operating Characteristics curve analysis showed that these two biomarkers had good sensitivity and specificity values in assisting the diagnosis of depressive disorders. N-methylnicotinamide has a sensitivity of 92.3% and a specificity of 100%, while hippuric acid has a sensitivity of 76.9% and a specificity of 84.6%. **Conclusions:** Significant differences in the biomarkers of N-methylnicotinamide and hippuric acid in the urine of depressed patients compared to normal patients. Therefore, these biomarkers can be the empirical laboratory methods to support the diagnosis of depressive disorders.

Keywords: Diagnostic, Depressive Disorder, Hippuric Acid, N-Methylnicotinamide, Mental Disorder

Cite this as: Susanto. N. A., Hasanah. U., et al. "Identification of Potential Diagnostic Markers for Depressive Disorders using Urinary Biomarkers of N-Methylnicotinamide and Hippuric Acid". Jurnal Psikiatri Surabaya, vol. 13, no. 2, pp.179-188, 2024. doi: 10.20473/jps.v13i2.47986

INTRODUCTION

The diagnosis of depressive disorders is based on the subjective identification of symptom clusters [1]. However, this method cannot guarantee adequate heterogeneity, so errors in diagnosing mental disorders are unavoidable. Shen et al. [2] explain that some mental disorders are often misdiagnosed, such as depression, bipolar, schizophrenia, anxiety, ADHD, and other disorders. A study by the Johns Hopkins Bloomberg School of Public Health (2013) [3] revealed that more than 60% of 5,600 patients got misdiagnosis of major depressive disorder. Besides, WHO (2021) states that there is often a misdiagnosis of depressive disorders, so antidepressants are often given [4]. Moreover, a study by the Royal College of Physicians in 2012 found that 85% of participants experienced a delay in diagnosis, which caused 71% of people to experience worsening symptoms [5]. Therefore, supporting accurate and objective diagnostic methods using empirical laboratories for patients with depressive disorders is important.

In its development, studies by Comes et al. [6], and Lakhan et al. [7] ound differences in blood levels of biomarkers in patients with severe mental illnesses such as schizophrenia, bipolar, and major depression. Bilello et al. [8] revealed that there were nine biomarkers in the blood to identify people with major depressive disorder with an objective accuracy of more than 90%. Besides, Cui et al. [9] found an RNA molecule in blood mononuclear cells that can be used as a new non-invasive biomarker for major depressive disorder. The difficulty in taking blood samples in research subjects with mental disorders becomes a challenge for future researchers [10]. Moreover, taking samples in blood tests can make some patients feel uncomfortable and nauseous, as some people have a phobia of needles or blood and require expensive examination fees [11]. Thus, other biomarkers that are easy to obtain, such as urine, are needed to support rapid and accurate early diagnosis as prevention, treatment, and prognosis of various diseases Astuti [12] states that urine is closely related to blood, as it is the residue in the blood that is filtered by the kidneys to maintain body fluid homeostasis. Holmen et al. [13] revealed that fluids and urine-forming materials came from blood or interstitial fluid. Thus, the use of urine as a liquid that is closely related to blood is predicted to also help in supporting the diagnosis of mental disorders, especially depression. Many studies concern the use of urine as a biomarker of mental illness; for example, Chen et al. [14] used organic acids in urine in combination with the XGBoost algorithm to be a new and accurate potential biomarker for the pathogenesis of autism. Zheng et al. [15] found that urine-based laboratory tests using urine biomarkers could be useful in the diagnosis of bipolar disorder. Thus, as a non-invasive biological fluid that is easily available, urine can be an important source for studies of disease biomarkers.

Urinalysis is an analysis method to find out the substances contained in the urine and the presence of abnormalities in the urine. Firdausa et al. [16] define urinalysis as the identification of urine macroscopically, microscopically, and chemically to help establish a diagnosis of kidney disease and other diseases. Qualitatively, urine examination aims to identify substances that are normally and abnormally present in urine. However, quantitatively or semi-quantitatively, the urine examination aims to determine the amount of these substances in the urine [17]. Harpole, et al. [18] revealed that urine was an excellent biospecimen for analyzing biomarkers because it can be collected repeatedly with non-invasive techniques and relatively large volumes. Therefore, urine is a potential biomarker for identifying depressive disorders in order to create an empirical laboratory method to support the diagnosis objectively, practically, and easily.

This present study used N-methylnicotinamide and hippuric acid biomarkers in urine as diagnostic supporting indicators for de-

pressive disorders because they are easily found in the results of the body's metabolism in urine. Holmen et al. [13] revealed that N-methylnicotinamide was a metabolite of niacin (nicotinamide) and commonly found in human urine. Many studies concern N-methylnicotinamide as a biomarker candidate for mental disorders such as bipolar disorder [19] and anxiety [1]. Zheng, et al. [19] tated that N-methylnicotinamide is involved in the tryptophan-nicotinic acid pathway, and an increase in N-methylnicotinamide in urine excretion indicates the regulation of tryptophan-nicotinic acid pathway activity in depressive disorder subjects. Meanwhile, hippuric acid is a carboxylic acid and an organic compound that can be found in urine. Referring to Raikhlin-Wisenkraft [20] the presence of a high hippuric acid biomarker in the urine indicates toluene poisoning, which can cause depression.

Based on the explanation above, a supporting method to empirically diagnose depressive disorders through laboratory tests for a faster, more precise, and more accurate analysis is important. Therefore, this study aims to identify the effectiveness of urine as a diagnostic support for depressive disorders using the N-Methylnicotinamide and Hippuric Acid biomarkers.

METHODS

This diagnostic and screening test research is an analytic observational study with a cross-sectional design. In this design, researchers observed and measured variables at certain times [21]. Putra, et al. [22] define a cross-sectional design in diagnostic testing and screening means that all variables, including the test and the gold standard, are measured in the same period to ensure that the disease conditions are still the same and valid.

This research was conducted for 4 months from June-September 2021 with ethical approval number E.5.a/160/KEPK-UMM/VI/2021. This study used 13 urine samples from depressed patients and 13 urine samples from non-depressed people (normal) aged 17-70 years. The determination of the sample used a purposive sampling technique with inclusion criteria of patients with depressive disorders and normal (without mental disorders) who had been diagnosed by a psychiatrist and were willing to have their urine taken. The exclusion criteria were patients with kidney disease who were not fasting or dieting before taking urine. The urine sample was taken at Muhammadiyah Lamongan Hospital, East Java, for depressed patients, and samples of non-depressed (normal) people were carried out in Malang City, East Java.

Samples of urine from depressed and non-depressed patients were determined by selecting subjects according to psychiatric standards through an anamnesis process based on the criteria for depressive symptoms in the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-V) and Guidelines for Diagnostic Classification of Mental Disorders III (PPDGJ). Besides, depression is also diagnosed through a special psychiatric examination for screening depressive disorders by filling in the Zung Self-Rating Depression Scale (SDS) by Zung [23], which has been adopted by Fadilah [24] with a reliability and validity value of 0.905 and 0.244-0.672. Each subject included in the inclusion criteria in both groups was required to collect 0.5 ml of urine samples.

This study used urine samples from 13 patients with depressive disorders and 13 people without depressive disorders (normal). Based on the demographic data, subjects with depressive disorder consisted of 3 patients with mild depressive disorder, 6 patients with moderate depressive disorder, and 4 patients with severe depressive disorder. The subject with depressive disorders consisted of 3 males and 10 females. Meanwhile, subjects without depressive disorders (normal) consisted of 5 males and 8 females. The tools and materials needed in this study were Standard Solution, plates, well strips, Biotinylated Human N-Methylnicotinamide

Antibody, Biotinylated Human Hippuric Acid Antibody, Sealer Plate, Stop Solution, Washing Buffer Concentrate, Streptavidin-HRP, substrate solution, incubator (37°C ± 0.5°C), absorbent paper, pipette, tube cleaner, deionized or distilled water, and microplate reader with wavelength filter of 450 ± 10nm.

N-methylnicotinamide and hippuric acid biomarkers were measured using the enzyme-linked immunosorbent assay (ELISA) method. ELISA is a plate-based assay technique designed to detect and quantify peptides, proteins, antibodies, antigens, glycoproteins, and hormones [25]. Its ability to wash non-specific and non-bonding materials makes the ELISA assay an accurate assay for measuring specific analytes [26]. The measurement of N-methylnicotinamide and hippuric acid biomarkers in urine used the ELISA kits according to the Elabscience instructions.

Data were analyzed using Mann-Whitney non-parametric hypothesis testing and ROC (Relative Operating Characteristics) analysis. The Mann-Whitney test was to check the significance difference between the means of two populations with the same distribution by taking two independent (free) samples from the two populations. The result is considered significant if the p-value is < 0.05, meaning that there is a significant difference between the biomarkers found in the urine of depressive disorder patients and non-depressed patients. Besides, the ROC analysis was to describe the accuracy of the diagnosis and determine the optimal cut-off value. The statistical tests used the SPSS program.

RESULTS

The results of the laboratory tests are presented; the mean value of the N-methylnicotinamide biomarker level of the depressed and non-depressed subjects reached 72.05 pg/ml and 3.99 pg/ml, respectively. Then, the mean value of the hippuric acid biomarker level of depressed and non-depressed subjects was 20.6 pg/ml and 11.5 pg/ml, respectively.

The Mann-Whitney test was also performed to compare the levels of N-methylnicotinamide and hippuric acid biomarkers using urine samples of depressed and non-depressed patients. The Mann-Whitney test is a non-parametric test to determine the difference in the median of 2 independent groups with no normal distribution. The result of the Mann-Whitney test showed significance values of 0.000 and 0.001 for the N-Methylnicotinamide biomarker and hippuric acid biomarker, respectively, with a p-value of <0.05 for both. Thus, there are significant differences between the N-Methylnicotinamide and Hippuric Acid biomarkers in the urine samples of patients with and without depressive disorders.

Besides, the Spearmans correlation test was conducted to determine the relationship between N-methylnicotinamide and hippuric acid biomarkers with depressive disorders. The results of the correlation test showed that there is a relationship between the two biomarkers for the sample group (depressed and non-depressed patients) in which the N-Methylnicotinamide biomarker has a stronger correlation (0.876) than the Hippuric Acid biomarker (0.692).

Diagnostic Performance Assessment

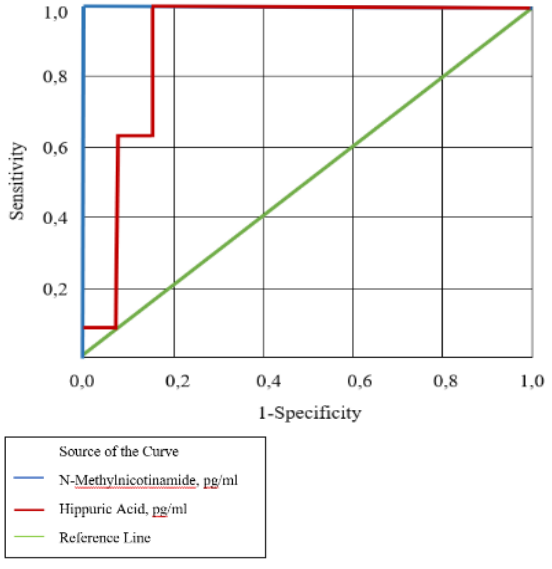

Figure 1. ROC Curve

Data were analyzed using the ROC (relative operating characteristics) curve to see the quality of the system in predicting depressive disorders. The curve can be seen in Figure 1 above. Based on the curve above, the AUC (Area Under the Curve) value of N-Methylnicotinamide is higher than that of Hippuric Acid. It is considered good if it is more than >80%-90%, and it is considered very good if it is more than >90%-100%. The results of the analysis of AUC are presented in Table 1.

Table 1. Area Under the Curve (AUC)

	Area	Std. Error[a]	Asymptotic Sig[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
[N-Methylnicotinamid], pg/ml	1.000	.000	.000	1.000	1.000
[Hippuric Acid], pg/ml	.899	.074	.001	.755	1.000

Based on the table above, the best determination of depression is N-methylnicotinamide levels. However, both biomarkers had significant AUC values ($p<0.05$). Besides, these two biomarkers show good sensitivity and specificity values, as indicated by the ROC cut points in Figure 2 and Figure 3 below.

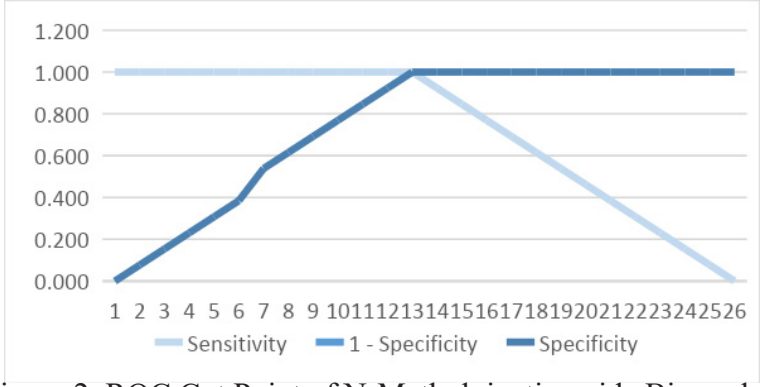

Figure 2. ROC Cut Point of N-Methylnicotinamide Biomarker

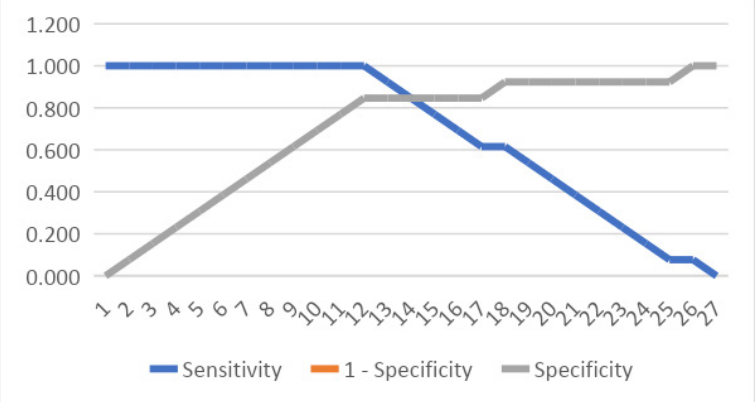

Figure 3. ROC Cut Points of Hippuric Acid Biomarkers

The cut-off result of the N-methylnicotinamide biomarker is ≥ 28.022 pg/ml with a sensitivity value of 92.3% and a specificity value of 100%. Meanwhile, the cut-off value for hippuric acid is ≥ 15.0635 pg/ml, with a sensitivity value of 76.9% and a specificity value of 84.6%. Thus, based on the ROC curve, the sensitivity and specificity of the biomarker cut-off are good. This means that

the higher the levels of N-methylnicotinamide and hippuric acid biomarkers in the urine, the higher the level of depression suffered. Therefore, N-methylnicotinamide and hippuric acid biomarkers can be further developed as indicators in supporting the diagnosis of patients with depressive disorders.

DISCUSSION

The results of this study support further discussion regarding the heterogeneity of biological conditions for depressive disorders through the identification of urine. This proves that urine is metabolism waste, which shows a link with depressive disorders. The results of this study are in line with Ilavska et al. [27] who found a disturbance in the process of neurotransmitter metabolism in urine in the etiopathogenesis (origin of disease based on etiology and pathogenesis) of depressive disorders. Besides, Chen et al. [28] revealed that the severity of depressive disorders is closely related to the presence of differential metabolites in the urine. Moreover, Chen et al. [29] also found that there are phenotype differences in urine metabolism in major depressive disorder and bipolar disorder. Zheng et al. [15] state that changes in urine metabolites indicate that disturbances in amino acid metabolism and energy metabolism, and intestinal microflora are associated with depressive disorders. The existence of a link between depressive disorders and urine leads to further review of the pathophysiology of depressive disorders. The use of urine provides significant results with the presence of differences in levels in groups with depressive disorders and normal (without depressive disorders). Chen et al. [1] state that most of the urine is a liquid that has very high value as a diagnostic biofluid so urine metabolites are often used to identify biomarkers of certain diseases. Besides, Jing & Gao [30] explain that urine as a non-invasive biological fluid and easy to obtain, is an important source for the study of disease biomarkers. The results of ROC analysis to determine the accuracy of the diagnosis by identifying the relationship between sensitivity and specificity show a good UAC value between the two biomarkers. Thus, this study proves that urine can be one of the biomarkers that can support the early diagnosis and prognosis of certain diseases, including depressive disorders. Recently, many researchers use metabolomics to study urine metabolites to identify disease biomarkers. Zheng, et al. [15] state that recently, there are 294 identified biomarkers in urine metabolites.

In this study, two metabolic platforms were carried out to explore metabolic changes in patients with depressive disorders and non-depressive disorders through urine. The identification was carried out on the metabolites of N-Methylnicotinamide and Hippuric Acid. Based on the results of the study, the levels of the two metabolites have a significant difference between the depressive and non-depressive groups, as indicated by higher levels of the two biomarkers in the depressive disorder group compared to the non-depressive group. In addition to the significant differences between the two biomarkers in the depressive and non-depressive groups, this study obtained good sensitivity and specificity values for N-Methylnicotinamide and Hippuric Acid biomarkers as diagnostic support for depressive disorders in the future. The increase in N-methylnicotinamide and hippuric acid biomarkers in the urine of the depressive disorder group is inseparable from a disturbance in the hypothalamus in the brain, which causes norepinephrine or noradrenaline to increase, thereby disrupting the body's metabolism.

The results of the analysis in this study are in line with previous studies that the metabolism of tryptophan, nicotinic acid, and N-Methylnicotinamide (NMNA) is the end product of nicotinamide metabolism, which increases significantly in subjects with major depression relative to health control (HC) [19]. Besides, Lester, et al. [31] found that the precursor nicotinamide, N-methyl-

nicotinamide, is involved in the disruption of the tryptophan-nicotinic acid pathway. Carney et al. [32] revealed that tryptophan is a biochemical precursor of serotonin and nicotinic acid. Thus, an increase in nicotinic acid metabolites may indicate a decrease in serotonin biosynthesis. This is also supported by Chen et al. [1] who found a significant change in N-methylnicotinamide levels associated with interference with tryptophan-nicotinic acid pathway activity in patients with depression and anxiety disorders. Lin et al. [33] ported that hippuric acid levels significantly changed in patients with postpartum depression. Hou et al. [34] found significant changes in hippuric acid levels in hepatitis patients with depression. In line with previous findings, changes in serotonergic neurotransmission may contribute to the pathophysiology of depression and anxiety disorders [35].

In this study, the Hippuric Acid biomarker also showed significant value in differentiating between the urine of groups with depressive disorders and non-depressive disorders. Chen, et al. define Hippuric Acid as a phenylalanine metabolite by intestinal microflora. The transamination effect of intestinal microflora on tryptophan and indoxyl sulfate results in [36]. Indole-3-acetate (IAA) containing Tryptophanase which is the result of tryptophan metabolism. Lesch, et al. [37] found that decreased IAA was associated with loss of appetite which is a common symptom in depressive disorders. Moreover, Cheung et al. [38] found that changes in intestinal microflora can cause gastrointestinal symptoms in depressed patients as well as comorbidities from major depression and irritable bowel syndrome. Hippuric Acid is believed to be a metabolite associated with disturbances in the gut microflora as it occurs in approximately two-thirds of patients with depressive disorders who describe somatic symptoms such as lack of energy or fatigue, pain, and gastrointestinal symptoms which are indicated as somatic symptoms in depressive disorders [39]. This consistently proves that depressive disorders will cause disturbances in the intestinal microflora and phenylalanine metabolism increasing the levels of the Hippuric Acid biomarker.

Based on the explanation above, the biomarkers explain the relationship between depression and somatic symptoms in urine metabolite levels, as evidenced by the higher levels of N-Methylnicotinamide and Hippuric Acid biomarkers in the urine, the higher the level of depression suffered. Therefore, the results of this study indicate that N-methylnicotinamide and hippuric acid biomarkers in urine can be used as biological markers for patients with mental disorders, especially patients with depressive disorders. The findings in this study also support N-methylnicotinamide and hippuric acid biomarkers in urine to support laboratory tests in the diagnosis of mental disorders, which were previously carried out using symptom clusters or non-laboratory tests. Laboratory tests using biomarkers in urine are recommended to support the results of analysis by experts (psychologists or psychiatrists) in determining mental disorders, especially depressive disorders, in order to obtain precise and accurate results. Thus, non-laboratory diagnostic methods using symptom clusters as the main diagnostic method supported by laboratory tests through the identification of this biomarker are needed.

The results of this study are expected to help create a kit that can help facilitate practitioners in effectively diagnosing patients with depressive disorders. Thus, it can be implemented directly and is marketable and useful as a tool to support diagnostics quickly and precisely for psychologists or psychiatrists. However, this study has some limitations in that the urine samples of depressed patients used came from depressed patients who had undergone treatment for more than two months. Moreover, uncontrolled diet intake still occurs in the subjects, so it can affect the levels of urine biomarkers. Urine

sampling from depressed and normal patients was not taken simultaneously because urine sampling for depressed patients was taken at RSM Lamongan and urine samples from normal subjects were taken in Malang. Thus, urine sampling was taken for more than 2 hours so that it could be a confounding factor in the results of the biomarker levels.

CONCLUSION

The results indicate that there are significant biological changes in urine due to psychological disorders, especially depression. The differences in N-Methylnicotinamide and Hippuric biomarkers also have good value in diagnostic studies as evidenced by their good sensitivity and specificity values in the accuracy of the diagnosis of depressive disorders through urine.

ACKNOWLEDGEMENT

The researcher highly appreciates the Directorate of Learning and Student Affairs of the Higher Education Directorate, Ministry of Education, Culture, Research and Technology of the Republic of Indonesia for funding the 2021 student creativity program.

CONFLICT OF INTEREST

All authors declare no conflict of interests.

FUNDING

No specific funding was provided for this study.

REFERENCES

[1] J. jun Chen et al., "Urinary biomarker panel for diagnosing patients with depression and anxiety disorders," Transl Psychiatry, vol. 8, no. 1, Dec. 2018, doi: 10.1038/s41398-018-0245-0.

[2] H. Shen, L. Zhang, C. Xu, J. Zhu, M. Chen, and Y. Fang, "Analysis of Misdiagnosis of Bipolar Disorder in An Outpatient Setting," Shanghai Arch Psychiatry, vol. 30, no. 2, pp. 93–101, Apr. 2018, doi: 10.11919/j.issn.1002-0829.217080.

[3] Johns Hopkins Bloomberg School of Public Health, "Over-diagnosis and over-treatment of depression is common in the U.S." Accessed: Sep. 29, 2023. [Online]. Available: https://publichealth.jhu.edu/2013/mojtabai-depression-over-diagnosis-and-over-treatment

[4] World Health Organization, "Depression." Accessed: Sep. 29, 2023. [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/depression

[5] C. Galletly et al., "Royal Australian and New Zealand College of Psychiatrists clinical practice guidelines for the management of schizophrenia and related disorders," Australian and New Zealand Journal of Psychiatry, vol. 50, no. 5. SAGE Publications Inc., pp. 410–472, May 01, 2016. doi: 10.1177/0004867416641195.

[6] A. L. Comes, S. Papiol, T. Mueller, P. E. Geyer, M. Mann, and T. G. Schulze, "Proteomics for blood biomarker exploration of severe mental illness: pitfalls of the past and potential for the future," Translational Psychiatry, vol. 8, no. 1. Nature Publishing Group, Dec. 01, 2018. doi: 10.1038/s41398-018-0219-2.

[7] S. E. Lakhan, K. Vieira, and E. Hamlat, "Biomarkers in psychiatry: drawbacks and potential for misuse," 2010. [Online]. Available: http://www.intarchmed.com/content/3/1/1

[8] J. A. Bilello et al., "MDDScore: Confirmation of a blood test to aid in the diagnosis of major depressive disorder," Journal of Clinical Psychiatry, vol. 76, no. 2, pp. e199–e206, Feb. 2015, doi: 10.4088/JCP.14m09029.

[9] X. Cui et al., "Long non-coding RNA: Potential diagnostic and therapeutic biomarker for major depressive disorder," Medical Science Monitor, vol. 22, pp. 5240–5248, Dec. 2016, doi: 10.12659/MSM.899372.

[10] M. L. Wainberg et al., "Challenges and Opportunities in Global Mental Health: a Research-to-Practice Perspective," Current Psychiatry Reports, vol. 19, no. 5. Current Medicine Group LLC 1, May 01, 2017. doi: 10.1007/s11920-017-0780-z.

[11] M. G. Meentken et al., "EMDR for children with medically related sub-threshold PTSD: short-term effects on PTSD, blood-injection-injury phobia, depression and sleep," Eur J Psychotraumatol, vol. 11, no. 1, Dec. 2020, doi: 10.1080/20008198.2019.1705598.
[12] T. I. Astuti, T. Ariyadi, and A. Sukeksi, "Perbedaan Jumlah Silinder Urin yang Diperiksa Segera," 2018. [Online]. Available: http://repository.unimus.ac.id
[13] H. Holmen, H. Egsgaard, J. Funck, and E. Larsen, "N'-Methylnicotinamide in Human Urine," 1981.
[14] Q. Chen, Y. Qiao, X. J. Xu, Y. Tao, and X. You, "Urine organic acids as potential biomarkers for autism-spectrum disorder in chinese children," Front Cell Neurosci, vol. 13, Apr. 2019, doi: 10.3389/fncel.2019.00150.
[15] S. Zheng et al., "Urinary metabonomic study on biochemical changes in chronic unpredictable mild stress model of depression," Clinica Chimica Acta, vol. 411, no. 3–4, pp. 204–209, Feb. 2010, doi: 10.1016/j.cca.2009.11.003.
[16] S. Firdausa, P. Pranawa, and S. D. Suryantoro, "Arti Klinis Urinalisis pada Penyakit Ginjal," 2018.
[17] R. M. Riswanto, "Pemeriksaan Kimia Urine," Pustaka Rasmedik, pp. 51–117, 2015.
[18] M. Harpole, J. Davis, and V. Espina, "Current state of the art for enhancing urine biomarker discovery," Expert Review of Proteomics, vol. 13, no. 6. Taylor and Francis Ltd, pp. 609–626, Jun. 02, 2016. doi: 10.1080/14789450.2016.1190651.
[19] P. Zheng et al., "Identification and validation of urinary metabolite biomarkers for major depressive disorder," Molecular and Cellular Proteomics, vol. 12, no. 1, pp. 207–214, Jan. 2013, doi: 10.1074/mcp.M112.021816.
[20] B. Raikhlin-Eisenkraft, E. Hoffer, Y. Baum, and Y. Bentur, "Determination of Urinary Hippuric Acid in Toluene Abuse," 2001. [Online]. Available: www.dekker.com
[21] A. Nurdini, "'CROSS-SECTIONAL VS LONGITUDINAL,'" 2006. [Online]. Available: http://www.petra.ac.id/~puslit/journals/dir.php?DepartmentID=ARS
[22] I. W. G. A. E. Putra, I. M. Sutarga, M. P. Kardiwinata, N. L. P. Suariyani, N. W. Septarini, and I. M. Subrata, Modul Penelitian Uji Diagnostik. Denpasar: Program Studi Kesehatan Masyarakat Fakultas Kedokteran Universitas Udayana Denpasar, 2016.
[23] W. W. K. Zung, "Chapter 21 Zung Self-Rating Depression Scale and Depression Status Inventory," Spinger, 1986.
[24] S. Z. Fadilah, "Hubungan dukungan keluarga dengan depresi penderita kusta di dua wilayah tertinggi kusta di Kabupaten Jember," Unveritas Jember, Jember, 2013.
[25] M. Alhajj and A. Farhana, "Enzyme linked immunosorbent assay," StatPearls, 2021.
[26] Boster Biological Technology, ELISA Handbook: Principle, Troubleshooting, Sample Preparation and Assay Protocols. 2020.
[27] L. Ilavská, M. Morvová Jr, J. Trebatická, Z. Ďuračková, and L. Šikurová, "Determination of metabolites in urine of youths with depression," in Book of Contributions 9th Slovak Biopshysical Symposium, 2020.
[28] J. jun Chen et al., "Differential urinary metabolites related with the severity of major depressive disorder," Behavioural Brain Research, vol. 332, pp. 280–287, Aug. 2017, doi: 10.1016/j.bbr.2017.06.012.
[29] J. J. Chen et al., "Divergent Urinary Metabolic Phenotypes between Major Depressive Disorder and Bipolar Disorder Identified by a Combined GC-MS and NMR Spectroscopic Metabonomic Approach," J Proteome Res, vol. 14, no. 8, pp. 3382–3389, Aug. 2015, doi: 10.1021/acs.jproteome.5b00434.
[30] J. Jing and Y. Gao, "Urine biomarkers in the early stages of diseases: current status and perspective," Discov Med, vol. 25, no. 136, pp. 57–65, 2018.
[31] G. Lester, "End-Product Regulation of the Tryptophan-Nicotinic Acid Pathway in Neurospora crassa," 1971. [Online]. Avail-

able: https://journals.asm.org/journal/jb
[32] D. R. Carney, A. J. C. Cuddy, and A. J. Yap, "Power Posing: Brief Nonverbal Displays Affect Neuroendocrine Levels and Risk Tolerance," Psychol Sci, vol. 21, no. 10, pp. 1363–1368, 2010, doi: 10.1177/0956797610383437.
[33] L. Lin, X. M. Chen, and R. H. Liu, "Novel urinary metabolite signature for diagnosing postpartum depression," Neuropsychiatr Dis Treat, vol. 13, pp. 1263–1270, May 2017, doi: 10.2147/NDT.S135190.
[34] L. J. Hou et al., "Urinary metabonomics for diagnosis of depression in hepatitis B virus-infected patients.," Iranian Red Crescent Medical Journal, , vol. 17, no. 4, 2015.
[35] D. Senkowski, M. Linden, D. Zubrägel, T. Bär, and J. Gallinat, "Evidence for Disturbed Cortical Signal Processing and Altered Serotonergic Neurotransmission in Generalized Anxiety Disorder," 2003.
[36] Y. Chen et al., "Yuanhuapine-induced intestinal and hepatotoxicity were correlated with disturbance of amino acids, lipids, carbohydrate metabolism and gut microflora function: A rat urine metabonomic study," J Chromatogr B Analyt Technol Biomed Life Sci, vol. 1026, pp. 183–192, Jul. 2016, doi: 10.1016/j.jchromb.2015.08.024.
[37] K. P. Lesch, J. Gross, E. Franzek, B. L. Wolozin, P. Riederer, and D. L. Murphy, "Primary Structure of the Serotonin Transporter in Unipolar Depression and Bipolar Disorder," 1995.
[38] S. G. Cheung, A. R. Goldenthal, A. C. Uhlemann, J. J. Mann, J. M. Miller, and M. E. Sublette, "Systematic review of gut microbiota and major depression," Frontiers in Psychiatry, vol. 10, no. FEB. Frontiers Media S.A., 2019. doi: 10.3389/fpsyt.2019.00034.
[39] X. Yuan et al., "Depression and anxiety in patients with active ulcerative colitis: crosstalk of gut microbiota, metabolomics and proteomics," Gut Microbes, vol. 13, no. 1, 2021, doi: 10.1080/19490976.2021.1987779.