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Role of Hepcidin in Pediatric Chronic Kidney Disease with Anemia

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ABSTRACT

Introduction: Anemia is a frequent complication of chronic kidney disease (CKD) in children and it causes an increase in morbidity, mortality and accelerates the rate of progression of CKD. Inflammation and impaired kidney clearance increase plasma hepcidin, inhibiting duodenal iron absorption and sequestering iron in macrophages. However, the role of hepcidin in increasing the risk of anemia in children with CKD is still uncertain. This study aimed to investigate the association between hepcidin levels and anemia in children with pre-dialysis CKD.

Methods: A cross-sectional study was conducted at Dr. Soetomo Academic Hospital from December 2018 to February 2019. Children with pre-dialysis CKD were enrolled in this study. The subject had no history of erythropoietin administration and blood transfusion 3 months before the blood sample were withdrawn. A complete blood count, ferritin serum, transferrin saturation (TSAT) and hepcidin serum were performed. The correlations between Hepcidin and ferritin level, between ferritin level and anemia, and between TSAT and anemia were analyzed using Spearman correlation and the Mann-Whitney test.

Results: A total of 47 children, 27 boys and 20 girls, ranged in age from 3 months to 18 years old. There was a significant correlation between hepcidin and ferritin levels (p=0.006) and the value of the Spearman correlation was r=0.392. While the correlation between ferritin level and anemia showed a significant result, p=0.001. However, TSAT did not show any significant correlation with anemia (p=0.230).

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Conclusion: There was an indirect association between hepcidin level and anemia by increasing ferritin level that induces anemia in pre-dialysis CKD children.

Keywords: Hepcidin; anemia; children; chronic kidney disease

Introduction

Anemia is highly prevalent in CKD and the severity increases as the disease advances and is associated with poor prognosis and increased mortality[1]. Data from the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) revealed the prevalence of anemia in children with CKD, ranging from 73 to 93%, depending on the CKD stages[1], [2]. While data from Dr. Soetomo Academic Hospital, Surabaya, Indonesia from January 2010 to February 2014 revealed the prevalence of anemia was 73.2%[3]. Anemia is an important risk factor for the development and progression to end-stage renal disease[1], [2].

Hepcidin is being extensively studied for its association with anemia in CKD where it has also been associated with inflammation. Hepcidin is thought to be the major regulator of dietary iron absorption and cellular iron release from macrophages, and it exerts its regulatory by function contracting the function of ferroportin, the major cellular iron exporter. Hepcidin induces internalization and degradation of ferroportin which results in increased intracellular iron stores, decreased dietary iron absorption and decreased circulating iron levels which may be the cause of functional iron deficiency (FID)[4]–[6]. Inflammation is a characteristic feature of CKD and is caused by multiple factors, such as the toxic uremic milieu and the dialysis procedure itself. The interpretation of iron biomarkers is hindered by inflammation, which can directly affect the concentrations of most iron biomarkers[7], [8], including ferritin and hepcidin[8]–[10]. Inflammation in CKD increases ferritin and hepcidin independent of iron status which reduces iron availability[9]. High hepcidin levels inhibit iron absorption from the gut and release from iron-storing cells, thus restricting erythropoiesis and leading to anemia[10], [11]. However, the role of hepcidin in increasing the risk of anemia in children with CKD is still uncertain. This study aimed to investigate the association hepcidin levels and anemia in children with pre-dialysis chronic kidney disease.

Methods

A cross-sectional study was conducted in Nephrology Division, Department of Child Health, Dr. Soetomo Academic Hospital, Faculty of Medicine Airlangga University, Surabaya-Indonesia from December 2018 to February 2019. Pre-dialysis patients with CKD from the age of 3 months to 18 years old were enrolled after obtaining informed consent from their parents. Patients having erythropoietin, blood

transfusion, supplementation of iron and acute infection within 3 months before the blood sample was withdrawn and excluded from the study.

CKD was defined according to the National Kidney Foundation/Kidney Disease Outcome Quality Initiative (NKF/KDOQI, 2003). CKD is defined as abnormalities of kidney structure or function of the kidney, with or without decreased GFR, or GFR < 60 ml/minute/1.73 m^2 for ≥ 3 months, with or without kidney damage.

The pre-dialysis patients that were included in the study had CKD with stage 1-5 pre-dialysis. Anemia was defined according to KDIGO Anemia 2012, diagnosis anemia in children with CKD if Hb concentration is <11.0 g/dL (< 11 g/dL in children 0.5-5 years, <11.5 g/dL in children 5-12 years, and < 12.0 g/dL in children 12-15 years), and anemia in children > 15 years with CKD when the Hb concentration is < 13.0 g/dL in males and < 12.0 g/dL in females.

Six ml of venous blood samples were drawn using fresh Ethylene diamine tetra acetic acid (EDTA). The serum and plasma were obtained after centrifugation for at least 15 minutes at 2200-2500 RPM within one hour of collection and then frozen at -80°C before laboratory analysis was done. Complete blood count, iron profile, creatinine, and urea were measured using standard laboratory methods (automated system) in a central laboratory. While, commercially available kits were used to measure hepcidin-25 (by sandwich enzyme-linked immunosorbent assay (ELISA) methods, human Hepc[Hepcidin] kit e-lab science biotechnology). The study was approved by the institutional Ethical Committee at Dr. Soetomo Academic Hospital (No. 0835/KEPK/XII/2018).

Baseline characteristics were assessed with standard descriptive statistics. The Kolmogorov-Smirnov test was used to determine the normalcy of the data. If normalcy was rejected, the nonparametric test was used. Continuous variables were presented as mean ± standard deviation (SD) and median with interquartile range (as applicable). Quantitative variables were determined using the Mann-Whitney test (for nonparametric data) between ferritin and anemia. The Spearman correlation coefficient was used to find a correlation between Hepcidin with ferritin levels, between ferritin levels with anemia and between TSAT with anemia. The data were entered into SPSS 17, and analysis was performed using statistics softwareStatistical significance was defined as $p<0.05$

Result

A total of 47 patients were enrolled in this study. The majority (38.2%) of the patients had lupus nephritis, nephrotic syndrome (23.4%), urology disorder (23.4%), tubulopathy (10.6%), and others (4.3%). In pre-dialysis CKD stages 1, 2, 3, 4, and 5, there were 16, 6, 12, 7, and 6 patients. Anemia occurred in 26 patients (55.3%) and without anemia in 21 patients (44.7%). Various parameters in pre-dialysis CKD in table 1. There was a significant correlation between hepcidin and ferritin level p=0.006(<0.05), the value of Spearman correlation coefficient was 0.392 (positive correlation). The ferritin level showed a significant

correlation with anemia, p=0.001(<0.05). However, TSAT did not show any significant relation with anemia p=0.230(>0.05).

Table 1. Various parameters in pre-dialysis chronic kidney disease

Parameters	CKD anemia n=26	CKD without anemia n=21
	Mean/IQR	Mean/IQR
Age (months)	135.6	163.8
Male:female (%)	53.8:46.2	61.9:38.1
Hemoglobin (g/dL)	9.9 (5.4-13.1)	13.7 (11.1-16.3)
Ureum (mg/dL)	35.0 (9-123)	15.5 (4-44)
Creatinine (mg/dL)	2.65 (0.49-17.18)	0.44 (0.24-11.9)
eGFR (mL/min/1.73 m^2)	84.4	109.8
Iron (ug/dL)	54.8	68.0
TIBC (ug/dL)	195.0	286.0
TSAT (%)***	35.6	25.8
Ferritin (ug/L)**	396.3 (3.17-1825.6)	78.8
Hepcidin (ng/mL)*	32.3 (2.94-137.7)	69.6 (3.0-1192.0)

p<0.05: Statistically significant
*Spearman correlation between hepcidin (ng/mL) and ferritin (ug/L) level in pre-dialysis CKD (p=0.006; r=0.392)
** Spearman correlation between ferritin level and anemia, (p=0.001)
***Spearman correlation between TSAT and anemia (p=0.230)
CKD chronic kidney disease, IQR interquartile range, eGFR estimated glomerular filtration rate, TIBC total iron-binding capacity, TSAT transferrin saturation.

Discussion

Anemia guidelines for CKD patients consider that TSAT and ferritin are important markers of anemia in CKD, and iron replacement is based on TSAT and serum ferritin levels[12]. Hepcidin has an important role to identify types of anemia in children. Anemia in CKD showed a high hepcidin level, while, various in TSAT, ferritin, and soluble transferrin receptor. In iron deficiency anemia showed a low level of hepcidin, TSAT, ferritin and an increase of soluble transferrin receptor[13].

Inflammation has been implicated in many complications in CKD including malnutrition, atherosclerosis, and decreased iron utilization[14]. Inflammation stimulates hepcidin expression through various mechanisme[15]. Pro-inflammation cytokines are increased in CKD[8], [9]. Pro-inflammation cytokines

such as IL-Iβ and IL-6 stimulate hepcidin expression via the Janus kinase (JAK)/signal transducer and

activator of the transcription 3 (STAT) pathway[15]. Inflammation induces another cytokine active B which stimulates the BMP-6/SMAD pathway synergic ally with IL-6 and STAT3, leading to hepcidin expression[16]. Endoplasmic reticulum (ER) stress associated with inflammation increases hepcidin by activating SMAD1/5/8[17], IL-6-dependent phosphorylated STAT3 and ER stress-activated transcription factor, cyclic AMP response element-binding protein H (CREBH), which bind and activate hepcidin promoter activity[9], [10]. Inflammation inhibits MT-2 by suppressing STAT5[18] and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) which antagonized LPS-induced hepcidin transcription via the interaction with hepatocyte nuclear factor 4α[10], leading to activation of hepcidin translation. Inflammation-induced IL-1β also activates hepcidin expression by inducing CCAAT enhancer-binding protein (C/EBP)δ in hepatocytes[10], [19]. Hepcidin is the key regulator of systemic iron homeostasis. Hepcidin leads to internalization and degradation of the iron exporter ferroportin, which is present on the cell surface of macrophages and enterocytes. Thus, hepcidin inhibits the release of iron by macrophages and attenuates the iron uptake in the gut[20].

In a study by Zaritsky et al, the median hepcidin level was noted to be 25.3 ng/mL in healthy pediatric control[21]. In another study by Ridha NR *et al.* Makassar, in 35 normal body weight children was obtained the median hepcidin level of about 16.1 ng/mL[22]. This study demonstrated that the hepcidin level increased in CKD with and without anemia (32.3 ng/mL vs 69.6 ng/mL). It is shown that the inflammation occurred in all groups.

This study demonstrates that serum hepcidin levels are positively correlated with serum ferritin. High hepcidin levels increase ferritin levels and induces anemia. Inflammation stimulates hepcidin expression through pro-inflammation cytokines. Furthermore, High hepcidin inhibits the release of iron storage in hepatocytes, macrophages and inhibits iron absorption from the gut, this causes an increase ferritin levels and leading to hypoferremia, that induces anemia [23]. Eleftheriadis et al were found that hepcidin is increased and correlated with ferritin but not with TSAT[24]. Similar results were observed in this study. Higher hepcidin levels were associated with an increased risk for incident anemia in children with CKD.

Conclusion

There was an indirect association between hepcidin level and anemia in which hepcidin increases ferritin level and induces anemia in pre-dialysis CKD children.

Conflict of Interest

Jusli Aras, Astrid Kristina Kardani, Risky Vitria Prasetyo, Ninik Asmaningsih Soemyarso, Mohammad Sjaifullah Noer, and I Dewa Gede Ugrasena declare that they have no conflict of interest this publication.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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References

1. M. A. Atkinson, K. Martz, B. A. Warady, and A. M. Neu, "Risk for anemia in pediatric chronic kidney disease patients: A report of NAPRTCS," *Pediatr. Nephrol.*, vol. 25, no. 9, pp. 1699–1706, 2010, doi: 10.1007/s00467-010-1538-6.
2. K. Lee *et al.*, "Anemia and Iron Deficiency in Children with Chronic Kidney Disease (CKD): Data from the Know-Ped CKD Study," *J. Clin. Med.*, vol. 8, no. 2, p. 152, 2019, doi: 10.3390/jcm8020152.
3. P. Saraswati *et al.*, "Anemia in children with chronic kidney disease," *Paediatr. Indones.*, vol. 54, no. 4 Supplement, p. 231, 2014.
4. S. K. Sonkar *et al.*, "Association of hepcidin and anemia in early chronic kidney disease," *Saudi J. Kidney Dis. Transpl.*, vol. 30, no. 2, pp. 315–324, 2019, doi: 10.4103/1319-2442.256838.
5. P. S. Suchdev *et al.*, "Assessment of iron status in settings of inflammation: Challenges and potential approaches," *Am. J. Clin. Nutr.*, vol. 106, pp. 1626S-1633S, 2017, doi: 10.3945/ajcn.117.155937.
6. A. Jairam *et al.*, "Iron status, inflammation and hepcidin in ESRD patients: The confounding role of intravenous iron therapy," *Indian J. Nephrol.*, vol. 20, no. 3, pp. 125–131, 2010, doi: 10.4103/0971-4065.70840.
7. J. Małyszko *et al.*, "New parameters in iron metabolism and functional iron deficiency in patients on maintenance hemodialysis," *Pol. Arch. Med. Wewn.*, vol. 122, no. 11, pp. 537–542, 2012, doi: 10.20452/pamw.1458.
8. L. Mercadel *et al.*, "The relation of hepcidin to iron disorders, inflammation and hemoglobin in chronic kidney disease," *PLoS One*, vol. 9, no. 6, pp. 1–7, 2014, doi: 10.1371/journal.pone.0099781.
9. E. Łukaszyk, M. Łukaszyk, E. Koc-Zórawska, J. Tobolczyk, A. Bodzenta-ŁUkaszyk, and J. Małyszko, "Iron status and inflammation in early stages of chronic kidney disease," *Kidney Blood Press. Res.*, vol. 40, no. 4, pp. 366–373, 2015, doi: 10.1159/000368512.
10. N. Ueda and K. Takasawa, "Impact of inflammation on ferritin, hepcidin and the management of iron deficiency anemia in chronic kidney disease," *Nutrients*, vol. 10, no. 9, 2018, doi: 10.3390/nu10091173.
11. K. K. Goyal *et al.*, "Hepcidin and proinflammatory markers in children with chronic kidney disease: A case-control study," *Clin. Nephrol.*, vol. 89, no. 5, pp. 363–370, 2018, doi: 10.5414/CN109132.
12. S. W. Lee *et al.*, "Serum hepcidin and iron indices affect anemia status differently according to the kidney function of non-dialysis chronic kidney disease patients: Korean cohort study for outcome in patients with chronic kidney disease (KNOW-CKD)," *Kidney Blood Press. Res.*, vol. 42, no. 6, pp. 1183–1192, 2017, doi: 10.1159/000485865.
13. G. D'Angelo, "Role of hepcidin in the pathophysiology and diagnosis of anemia," *Blood Res.*, vol. 48, no. 1, pp. 10–15, 2013, doi: 10.5045/br.2013.48.1.10.
14. G. C. Reddy, R. Devaki, and P. Rao, "Iron Indices in Patients with Functional Anemia in Chronic Kidney Disease.," *Ejifcc*, vol. 24, no. 3, pp. 129–36, 2013, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/27683448%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4975187.
15. N. Zhao, A. Zhang, C. A. Enns, N. Zhao, A. Zhang, and C. A. Enns, "Iron regulation by hepcidin Find the latest version : Science in medicine Iron regulation by hepcidin," *J. Clin. Invest.*, vol. 123, no. 6, pp. 2337–2343, 2013, doi: 10.1172/JCI67225.SMAD5.
16. T. Ganz, "Systemic iron homeostasis," *Physiol. Rev.*, vol. 93, no. 4, pp. 1721–1741, 2013, doi: 10.1152/physrev.00008.2013.
17. S. Canali, C. Vecchi, C. Garuti, G. Montosi, J. L. Babitt, and A. Pietrangelo, "The SMAD pathway is required for Hepcidin response during endoplasmic reticulum stress," *Endocrinology*, vol. 157, no. 10, pp. 3935–3945, 2016, doi: 10.1210/en.2016-1258.
18. M. de la C. Ruiz-Jaramillo *et al.*, "Iron overload as cardiovascular risk factor in children and adolescents with renal disease.," *Nephrol. Dial. Transplant*, vol. 26, no. 10, pp. 3268–3273, 2011, doi: 10.1093/ndt/gfr044.
19. Y. Kanamori, M. Murakami, M. Sugiyama, O. Hashimoto, T. Matsui, and M. Funaba, "Interleukin-1β (IL-1β) transcriptionally activates hepcidin by inducing CCAAT enhancer-binding protein δ (C/EBPδ) expression in hepatocytes," *J. Biol. Chem.*, vol. 292, no. 24, pp. 10275–10287, 2017, doi: 10.1074/jbc.M116.770974.
20. D. W. Swinkels and J. F. M. Wetzels, "Hepcidin: A new tool in the management of anaemia in patients with chronic kidney disease?," *Nephrol. Dial. Transplant.*, vol. 23, no. 8, pp. 2450–2453, 2008, doi: 10.1093/ndt/gfn267.
21. M. A. Atkinson, J. Y. Kim, C. N. Roy, B. A. Warady, C. T. White, and S. L. Furth, "Hepcidin and risk of anemia in CKD: a cross-sectional and longitudinal analysis in the CKiD cohort," *Pediatr. Nephrol.*, vol. 30,

no. 4, pp. 635–643, 2015, doi: 10.1007/s00467-014-2991-4.
22. N. R. Ridha and D. Daud, "Hubungan Kadar Hepcidin dengan Status Besi pada Inflamasi Akibat Obesitas," *Sari Pediatr.*, vol. 16, no. 3, p. 161, 2016, doi: 10.14238/sp16.3.2014.161-6.
23. T. Ganz and E. Nemeth, "Iron balance and the role of hepcidin in chronic kidney disease," *Semin Nephrol.* 2016 March; 36(2): 87 - 93. doi:10.1016/j.semnephrol.2016.02.001.
24. T. Eleftheriadis *et al.*, "Ferroportin in monocytes of hemodialysis patients and its associations with hepcidin, inflammation, markers of iron status and resistance to erythropoietin," *Int. Urol. Nephrol.*, vol. 46, no. 1, pp. 161–167, 2014, doi: 10.1007/s11255-013-0497-9.

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Role of Knowledge, Subjective Norms, Perceived Behavior Control, and Attitudes in Predict Pro-Environmental Behavior

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ABSTRACT

Background: One of the public universities as the green campus that prioritizes reforestation to overcome environmental issues. All stakeholders including students are expected to behave pro-environmentally.This study aimed to explain whether or not there is an influence of knowledge, subjective norm, perceived behavior control, and attitude towards pro-environmental behavior (plastic waste) for university students.
Methods: This research was conducted under a quantitative approach with 5 valid and reliable variables. In addition, regression tests and structural equation models are used. The respondents of this study were 399 university students from all faculties of Hasanuddin University.
Results: Perceived Behavior Control has the greatest effect on pro-environmental behavior compared to other variables. However, environmental knowledge has the smallest effect on subjective norms, perceived behavior control, and attitude.
Conclusion: All variables affect predict pro-environmental behavior.

Keywords: University student; knowledge; subjective norms; perceived behavior control attitude

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Introduction

One thing that gets the spotlight on students and environmental problems is plastic waste. The number of students and activities affect the use of plastic waste. The number of students has the potential to increase the amount of plastic waste. Individuals should develop pro-environmental behavior in their lives, so that they are able to maintain the sustainability of the ecosystem. Pro-environmental behavior is another form of pro-social behavior. This is shown by individuals to reduce negative impacts on the environment caused by activities to achieve needs or in other words to improve environmental quality [1,2].

There are several obstacles explained that in developing pro-environmental behavior [3]. First, there is a difference between personal *interest (individual interest)* and social desire *(collective interest)*. *Collective interest* directs individuals to prioritize the environment. This is based on the view of the environment as something that needs to be taken care of together. One way is to consume pro-environmental products. However, *individual interest is* not like that. *Individual interest* prioritizes personal comfort over the environment. This causes individuals to tend to use products that are not pro-environmental, which has a negative impact on the environment.

To predict pro-environmental behavior, some experts have looked with *Theory of Planned Behavior*. TPB to examine the antecedents of pro-environmental behavior [4,5,6]. TPB is used to explain motivational factors in individual behavior [7,8]. The theory proposes that human behavior is determined by behavioral intentions. Behavioral intentions are influenced by *attitude*, *subjective norm*, and *perceived behavioral control* [9]. All of these variables are examined to determine the appropriate intervention, so that pro-environmental behavior can be improved. Furthermore, environmental preservation can also be achieved. However, the research results obtained are different.

Pro-environmental behavior is influenced by attitudes. That attitude is an individual's positive or negative evaluation of an object [10]. This makes attitudes can affect behavior. Generally, when individuals get information, their attitudes will change and that makes their behavior also change. This is supported by another research that attitudes influence on the behavior of reducing waste production in the Terban community. Individuals who obtain information about the environment and are supported by a positive attitude will behave to reduce waste production [11].

The *subjective norm* has a role in pro-environmental behavior. *Subjective norm* is a personal perception of a behavior that is influenced by others [12,13]. *Subjective norms* increase the likelihood of recycling for people who exhibit positive experiential attitudes. Subjective norms motivate people with limited knowledge about the benefits of recycling to practice recycling behavior [14].

Perceived control behavior has an important role in pro-environmental behavior. Perceived control

behavior is the individual's perception of his ability to engage in certain behaviors [15]. Individuals can behave in a certain way when there is such a perception in them [4]. The behavior management process is assumed to be substantially leading to action [16].

In addition to these three variables, the influence of *knowledge is* also calculated for its influence on these three variables and also pro-environmental behavior. The results shown are different. Based on the results of research by Abrauw, Yunus, and Giyarsih (2011) in Papua, it is explained that the level of behavior in processing inorganic waste in Papua is moderate (95%) [17]. Whereas the Papuan people have sufficient knowledge about environmental conditions through education organized by the government. However, this knowledge does not encourage individuals to behave pro-environmentally. Based on this research, one of the factors that influence knowledge that does not have a significant impact on pro-environmental behavior is the low score of attitudes and habit factors owned by the subjects in the study. This study aims to determine the effect of Knowledge, Subjective Norm, Perceived Behavior Control, and Attitude towards Pro-Environmental Behavior (Plastic Waste) on university students. The practical benefits of this research are as follows:

Find a model of the relationship between variables related to PEB for University students that can be used as the basis for policies (social intervention) from the University Leaders (Rector) or Faculty Leaders (Deans) to develop PEB for University students that support the creation of a beautiful campus environment "Green Campus" which supported by "Green Behavior" from University students in particular and the academic community in general. For individuals, as a consideration in behavior that can have an impact on the environment.

Methods

The type of research used is quantitative research. This type of quantitative research was chosen by considering that quantitative research can collect objectively measurable data based on the research variables to be studied, namely *knowledge*, *subjective norms*, *perceived behavior control*, and *attitude*, as well as pro-environmental behavior (plastic waste).

The population of this study came from all students of Hasanuddin University who are actively studying. Based on the population, the researcher will take part of the population to be sampled in this study. The number of samples of this research is 399 samples. This number has met the results of the sample size estimation through the application *G*Power* 3.1, named 89 samples with a *medium effect size* of 0.15, *error probability* of 0.05, the *statistical power* of 0.95. In addition, the number of samples of 200 is considered sufficient to test the validity and reliability. This research lasted for three months.

Techniques of Data Collection

Knowledge

The measuring tool knowledge consists of 10 true and false statement items related to plastic waste compiled by the authors. Participants were asked to determine whether the statement was true or not. Each question is based on scientific sources. If a participant answers correctly for a true statement, then the participant will get a value of 1. Conversely, if there are participants who answer incorrectly for a true statement, then the participant will get a value of 0.

Through exploratory factor analysis, 8 items were declared valid and 2 items were declared invalid because <0.03. The eight items have validity ranging from 0.659-0.815. In addition, the six items have the reliability of 0.848, so it can be said that the reliability is >0.5.

Tabel.1
Explanatory Factor Analysis

Aitem	***Factor loading***
Aitem 1	0.724
Aitem 2	0.795
Aitem 4	0.659
Aitem 5	0.712
Aitem 6	0.780
Aitem 7	0.815
Aitem 9	0.785
Aitem 10	0.804

N=399 (male=90; female=309); Factor Loading=large correlation between the indicator and its latent construct

Subjective Norm

The measuring tool is a subjective norm composed of 6 statements compiled by Zainal & Hassan (2019). Participants are welcome to choose the answer strongly disagree, disagree, neutral, agree, and strongly agree. Through confirmatory factor analysis, 4 items were declared valid and 2 items were declared invalid because <0.03. The four items have validity ranging from 0.560-0.693. In addition, the six items have reliability of 0.721, so that it can be said to be reliable > 0.5.

Tabel.2
Confirmatory Factor Analysis

Items	***Standardized Loading Factor***
Aitem 1	0.621
Aitem 2	0.693
Aitem 5	0.560
Aitem 6	0.637

N=399 (male=90; female=309); Factor Loading=large correlation between the indicator and its latent construct

Perceived Behavior Control

The measurement tool for perceived behavior control is composed of 6 statements compiled by Zainal & Hassan (2019). Participants are welcome to choose the answer strongly disagree, disagree, neutral, agree, and strongly agree. Through confirmatory factor analysis, 5 items were declared valid and 1 item was declared invalid because <0.03. The five items have validity ranging from 0.381-0.554. In addition, the six items have reliability of 0.496, so it can be said that the reliability is >0.5.

Tabel 3
Confirmatory Factor Analysis

Items	*Standardized Loading Factor*
Aitem 1	0.554
Aitem 2	0.435
Aitem 4	0.451
Aitem 5	0.381
Aitem 6	0.547

N=399 (male=90; female=309); Factor Loading=large correlation between the indicator and its latent construct

Attitude

The measurement tool is *attitude* composed of 15 statements Zainal & Hassan (2019). Participants are welcome to choose the answer strongly disagree, disagree, neutral, agree, and strongly agree. Through *confirmatory factor analysis*, 11 items were declared valid and 4 items were declared invalid because <0.03. The five items have validity ranging from 0.384-0.550. In addition, the six items have reliability of 0.810, so it can be said that the reliability is >0.5.

Tabel.4
Confirmatory Factor Analysis

Items	*Standardized Loading Factor*
Aitem 1	0.673
Aitem 2	0.514
Aitem 3	0.331
Aitem 4	0.533
Aitem 5	0.392
Aitem 6	0.486
Aitem 7	0.453
Aitem 8	0.729
Aitem 9	0.500
Aitem 10	0.457
Aitem 11	0.514

N=399 (male=90; female=309); Factor Loading=large correlation between the indicator and its latent construct

Pro-Environmental Behavior (Less Use of Plastic Waste)

The measuring tool for pro-environmental behavior is composed of 8 statements compiled by the

authors. Participants are invited to choose never, rarely, sometimes, often, and always based on statements regarding the behavior of using plastic waste.

Through *confirmatory factor analysis*, 6 items were declared valid and 3 items were declared invalid because <0.03. The eight items have validity ranging from 0.330 to 0.700. In addition, the six items have reliability of 0.848, so it can be said that the reliability is >0.5.

Tabel.5
Confirmatory Factor Analysis

Items	Factor Loading
Item 1	0.117
Item 2	0.330
Item 3	0.354
Item 4	0.641
Item 5	0.700
Item 6	0.517

N=399 (male=90; female=309); Factor Loading=large correlation between the indicator and its latent construct;

Data Analysis

Descriptive Analysis

This study uses descriptive analysis to obtain a description of the state of the variables to be studied and matters relating to these variables based on the data obtained. Data in the form of numbers will be processed by adding, comparing, and so on until a percentage is obtained. Furthermore, the data is interpreted in the form of a conclusion sentence.

Hypothesis Test

The hypothesis in this study will be tested using correlation analysis techniques. Statistical analysis techniques used are multiple regression and linear regression. As for testing the hypothesis using the help of SPSS 23.0 for windows

Result

In this study, the researcher first tested the assumptions. The assumption test consists of residual normality, linearity, heteroscedasticity, and multicollinearity tests. The residual normality test was obtained through the Kolmogorov-Smirnov value of 0.200. This means that the data is normally distributed.

The linearity test was obtained through knowledge, subjective norm, perceived behavior control, subjective norm, and attitude towards pro-environmental behavior (plastic waste). Overall it can be said to be linear because the value is linearity <0.05 and the deviation from linearity is >0.05

Heteroscedasticity test (Glejser) obtained by knowledge, *subjective norm, perceived behavioral control, subjective norm, and* attitude towards environmentally friendly behavior (plastic waste). In general, it can be said that there is no heteroscedasticity problem because the significance value is > 0.05.

Table.6
Heteroscedasticity test

Variable	Significance
Knowledge	0.868
Subjective Norm	0.395
Perceived Control Behavior	0.436
Attitude	0.052

Multicollinearity test obtained by knowledge, *subjective norm, perceived behavioral control, subjective norm, and* attitude towards environmentally friendly behavior (plastic waste). In general, it can be said that there is no multicollinearity problem because the *tolerance value is* < 1,000 and VIF < 10,000.

Tabel.7
Multicollinearity test

Variable	*Tolerance*	VIF
Knowledge	0.981	1.019
Subjective Norm	0.643	1.555
Perceived Control Behavior	0.696	1.436
Attitude	0.753	1.327

Tebel.8

Regression Test

Independent Variable	Dependent Variable	*R Square*	Asymp. sig
Knowledge	Pro-environmental behavior (Plastic Waste)	0.017	0.009
Subjective Norm	Pro-environmental behavior (Plastic Waste)	0.140	0.000
Perceived Behavior Control	Pro-environmental behavior (Plastic Waste)	0.183	0.000
Attitude	Pro-environmental behavior (Plastic Waste)	0.117	0.000
Knowledge, subjective norm, perceived behavior control, and attitude	Pro-environmental behavior (plastic Waste)	0.243	0.000

N=399 (male=90; female=309);R Square= coefficient of determination that can explain how far the dependent data can be explained by independent data.

Based on the results of the linear regression test, it can be seen that each independent variable can

affect the dependent variable. It is because the value of Asymp.sig <0.05. The magnitude of the influence between variables can be observed through R Square. Knowledge has the smallest effect on pro-environmental behavior (plastic waste), compared to subjective norms, perceived behavior control, and attitude, which is 0.017 or 1.7%. On the other hand, perceived behavior control has the greatest influence on pro-environmental behavior (plastic waste), which is 0.183 or 18.3%,

Tabel.9
Structural Equation Modeling

Path	Path coefficient	p-value	z-value
Perceived Behavior Control - Pro-environmental Behavior	0.222	0.000	4.285
Attitude - Pro-environmental Behavior	0.352	0.000	7.015
Perceived Behavior Control - Attitude	0.326	0.000	9.142
Subjective norm - Attitude	0.553	0.000	17.670
Subjective norm -Perceived Behavior Control	0.463	0.000	12.526

N=399 (male=90; female=309); Path Coefficient=the direct effect of the variable determined as the cause on the variable determined as the effect; p-value=significance level; z-value=determine the number of standard deviations above or below the mean

Tabel.10
The Goodness of Fit Model

Goodness of Fit Model	Value
P-Value of Chi-Square	0.181
RMSEA	0.044
NFI	0.996
CFI	0.998
GFI	0.997
AGFI	0.977
SRMR	0.012

N=399 (male=90; female=309); value=measure the accuracy of the sample regression function in estimating the actual value

Discussion

Through the results obtained in linear regression, it can be said that environmental knowledge (plastic waste), subjective norms, perceived behavior control, and attitude affect pro-environmental behavior. However, environmental knowledge has the smallest effect on subjective norms, perceived behavior control, and attitude. In addition, perceived has the greatest effect on pro-environmental behavior

compared to other variables.

These results are in line with the explanation that subjective norms, perceived behavior control, and attitude affect pro-environmental behavior based on TPB [9,15,18]. Perceived behavior control has a significant impact on commitment across various actions [19,20,21]. The results of this study show different results from research by Aziz et al., (2021) that the attitude of university employees directly affects the intention to behave in pro-environmental behavior. Human attitudes are strong predictors of subsequent behavior [22]. This notion also applies to the study of environmental behavior [23]. In this study, attitude is not the variable with the greatest influence, but perceived behavior control.

Furthermore, the effect of knowledge on pro-environmental behavior is the smallest. This is also explained through the research of Ajzen et al. (2011) that good information is a prerequisite for effective action to produce the desired results. In Study 1 (N = 79), environmental knowledge did not affect energy conservation, and in Study 2 (N = 79), alcohol knowledge was not associated with drinking behavior. Such disappointing correlations can result from an inappropriate focus on the accuracy of the information at the expense of its relevance and support for behavior. Study 3 (N = 85) found a positive correlation between knowledge and pro-Muslim behavior, but Study 4 (N = 89) confirmed the proposition that this correlation arises because responses on knowledge tests reflect underlying attitudes. Study 4 also shows that the correlation can be positive or negative by selecting the right questions for the knowledge test. The theory of planned behavior, with a focus on specific actions, intentions, and predicted behavior in all four studies [7].

Conclusion

This research was conducted on several variables that influence the behavior of using plastic waste, which are knowledge, subjective norms, perceived behavior control, and attitudes. Environmental knowledge (plastic waste), subjective norms, perceived behavior control, and attitude affect pro-environmental behavior. Perceived Behavior Control has the greatest effect on pro-environmental behavior compared to other variables. However, environmental knowledge has the smallest effect on subjective norms, perceived behavior control, and attitude.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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References

[1] Corral-Verdugo V, Frías M, & García C. Psychological approaches to sustainability. Nova Science Publishers;2010.
[2] Sawitri DR, Hadiyanto H, & Hadi SP. Pro-environmental behavior from a social cognitive theory perspective. Procedia Environmental Sciences [internet]. 2010;*23*:27–33.
[3] Steg L & De Groot JIM. The oxford handbook of environmental and conservation psychology. Oxford University Press;2012.
[4] Greave M, Zibarras L. D. & Stride C. Using the theory of planned behavior to explore environmental behavioral intentions in the workplace. Journal of Environmental Psychology [internet]. 2013;*34*:109-120.
[5] Oreg S & Katz-Gerro T. Predicting proenvironmental behavior cross-nationally: Values, the theory of planned behavior, and value-belief-norm theory. *Environment and Behavior [internet]*. 2006;*38(4)*:462–483.
[6] Paul J, Modi A, & Patel J. Predicting green product consumption using theory of planned behavior and reasoned action. Journal of Retailing and Consumer Services [internet]. 2016;*29:*123-134.
[7] Ajzen I, Joyce N, Sheikh S, & Cote NG. Knowledge and the prediction of behavior: The role of information accuracy in the theory of planned behavior. Basic and Applied Social Psychology [internet]. 2011;*33*:101-117.
[8] Madden TJ, Ellen PS, & Ajzen I. A comparison of the theory of planned behavior and the theory of reasoned action. *Personality and Social Psychology Bulletin [internet]*. 1992;*18(1)*:3–9.
[9] Ajzen, I. *Handbook of theories of social psychology*. Sage Publications Ltd;2012.
[10] Schultz PW & Estrada-Hollenbeck M. Social Pyschology. Cambridge University Press;2008.
[11] Akhtar H &Soetjipto HP. Peran sikap dalam memediasi pengaruh pengetahuan terhadap perilaku minimisasi sampah pada masyarakat terban yogyakarta. Jurnal Manusia dan Lingkungan [internet]. 2014;*21(3)*:386-392.
[12] Ajzen I & Fishbein M. Understanding attitudes and predicting social behavior englewood cliffs. Prentice-Hall;1980.
[13] Fishbein M & Ajzen I. Belief, attitude, intention, and behavior: An introduction to theory and research, reading. Addison-Wesley;1975.
[14] Wan C, Shen GQ, & Choi S. Experiential and instrumental attitudes: Interaction effect of attitude and subjective norm on recycling intention. Journal of Environmental Psychology [internet]. 2017;*50*:69-79.
[15] Ajzen I. The theory of planned behavior. Organizational Behavior and Human Decision Processes [internet]. 1991;*50(2)*:179-211.
[16] Veronese D & Kensler L. School leaders, sustainability, and green school practices: An elicitation study using the theory of planned behavior. Journal of Sustainability Education [internet]. 2013;*4*:1-21.
[17] Abrauw AES, Yunus HS, & Giyarsih SR. Perilaku masyarakat dalam pengelolaan sampah anorganik di kecamatan abepura kota jayapura. Majalah Geografi Indonesia [internet]. 2011;*23(1)*:1-14.
[18] Ajzen, I. Perceived behavioral control, self-efficacy, locus of control, and the theory of planned behavior. *Journal of Applied Social Psychology [internet]*. 2002;*32(4)*, 665–683.
[19] Balderjahn I. Personality variables and environmental attitudes as predictors of ecologically responsible consumption patterns. *Journal of Business Research [internet]*. 1988;*17(1)*:51–56.
[20] Schwepker CH & Cornwell TB. An examination of ecologically concerned consumers and their intention to purchase ecologically packaged products. Journal of Public Policy & Marketing [internet]. 1991;10(2):77-101.
[21] Sparks P & Shepherd R. Self-identity and the theory of planned behavior: Assessing the role of identification with "green consumerism". *Social Psychology Quarterly [internet]*. 1992;*55(4)*:388–399.
[22] Aziz F, Rami AAM, Zaremohzzabieh Z, & Ahrari S. Effects of emotions and ethics on pro-environmental behavior of university employees: A model based on the theory of planned behavior. sustainability [internet]. 2021;*13(3)*:7062.
[23] Jakucionyte-Skodiene M, Dagiliute R, & Liobikiene G. Do general pro-environmental behaviour, attitude, ad knowledge contributeto energy saving and climate change mitigationin the residential sector. Journal Pre-proof [internet]. 2020;*193*:1-18.

REVIEW ARTICLE Open Access

Isolation and Identification of Bacteria in the Mouth Before and After Ablution

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ABSTRACT

Background: The oral is the gateway for the entry of various kinds of microorganisms into the body, with the prevalence of people having dental and oral problems in Indonesia increasing every year. The normal flora of the oral acts as a body defense, but it can cause disease due to predisposing factors, namely oral hygiene. Therefore, it is necessary to find an alternative in maintaining oral health. Islam is a religion that emphasizes personal hygiene, such as performing ablution.
Content: The types of bacteria found in the oral before ablution was 33.33% *Pseudomonas* sp., 6.67% *Lactobacillus* sp., 3.33% *Streptococcus* sp. and 0.14% *Staphylococcus* sp. while the types of bacteria found in the oral after ablution were 26,8% *Pseudomonas* sp., 20% *Lactobacillus* sp., 5% *Streptococcus* sp. and 2% *Staphylococcus* sp.
Conclusion: There was a change in the number of bacteria, namely an increase in gram-positive bacteria in the oral after ablution.

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Introduction

Health is the most important part of human life, which is physically and spiritually healthy. Health that needs to be considered in addition to general body health, is also dental and oral. Dental and oral health can affect overall body health because dental and oral health is an integral part of overall body health that cannot be separated from general body health.(1)

Based on the Basic Health Research (RIKESDAS) in 2018, the percentage of the population who had dental and oral problems in Indonesia had quite increased from the previous year, which was around 57.6%. By province which Having these problems, South Sulawesi ranks second out of the total.(2)

The mouth and nose are nests of harmful bacteria, if you don't clean them regularly, they will cause various diseases. The bacteria will be more fertile by the remaining food debris that is between the teeth and is not cleaned. However, not all microorganisms are pathogenic in the oral cavity, microorganisms that enter will be neutralized by anti-bacterial substances and normal flora bacteria. However, the presence of normal flora is not always beneficial, under certain conditions normal flora can cause disease, for example, if there is a change in substrate or displacement from the proper habitat. Research has proven that 90% of those who suffer from tooth decay are caused by mistakes in cleaning the mouth. Therefore, it is necessary to find alternatives in maintaining oral health.(5)(6)

Islam is a religion that always emphasizes personal hygiene before worship. This can be seen from the emphasis on bathing and ablution before performing obligatory worship, such as prayer. So Muslims must always maintain cleanliness because cleanliness will manifest physical and spiritual health. As a form of self-cleaning, when we perform ablution, we are also advised to rinse our mouth and siwak (toothbrush), clean the nose and, clean between the beards, as well as the fingers and toes.(7)(8)

Wudhu is a cleaning process carried out by a person to wash the body parts five times a day. According to research conducted by Herika Laksmi Safitri K. with the title "Isolation, Identification and Sensitivity Test of Antibiotic Germs in the Mouth Before and After Wudhu on Students of the 2016 Faculty of Medicine, Muslim University of Indonesia" concluded that there was a decrease in the number of gram-negative bacteria in the mouths of students after ablution. So that there will be a difference between students who have performed ablution and those who have not. Where the development of bacteria is more inhibited after ablution.(9)(10)

By looking at various facts regarding the benefits of ablution to public health, this research was carried out to prove and examine how the development of bacteria in the mouth after ablution and knowing the types of bacteria found in the oral cavity.

Content

A study conducted by Armiati, IGK in 2018 discussed the decrease in the number of *Streptococcus mutans* colonies in the oral cavity by ethanol extract of aloe vera leaf skin. The sample in this study was obtained by isolating oral bacteria where people tried to gargle with distilled water for 60 seconds without swallowing and the results of the mouthwash were discarded.(23)

Samples were taken using the swab method, where a sterile cotton swab was rubbed on the teeth on the labial surface of the upper right first incisor, the labial surface of the lower left first incisor, the buccal surface of the upper right first molar, the buccal surface of the upper left first molar, and the lingual surface. The lower left first molar and the lingual surface of the lower right first molar. This swab is done before and after gargling. The swab results were inserted into TSB media and cultured on Mueller Hinton Blood media. The culture results were seen after 24 hours and counted the number of bacterial colonies that grew and identified the bacteria by testing the bacteria with the Gram stain test, catalase test and mannitol test. So that the results obtained the number of *Streptococcus mutans* bacteria before gargling was 6114.80±2733.93 CFU/ml and after gargling was 3683.34±921.63 CFU/ml. This proves that there is a decrease in *Streptococcus mutans* bacteria after gargling with distilled water. At the same time, Ernawati, K. L (2018) conducted a study discussing kombucha tea, which can reduce the number of *Streptococcus mutans* bacteria in caries patients in 2019. Djohari et al conducted a study that discussed the identification and test of the inhibitory activity of ethanol extract areca nut (*Areca catechu L.*) against gum bacteria isolates. Where, in these two studies, sampling was carried out in the same way, namely by using a swab technique and placing it on the culture medium, then gram staining and biochemical tests were carried out to identify bacteria.(23)(24)(25)

In addition, a study by Da-Young et al in 2017, compared the acidity of the oral pH of each study subject, the patient's hygiene performance index score, and the number of bacteria in the oral cavity of each study subject before and after gargling and brushing teeth. In this study, 10 ml of saliva was taken from each subject, after that, it was diluted with agar, a mixture of Sigma Aldrich and agar powder (Samjeon Chemistry), with distilled water 7 times, and mixed with superior agar medium. The mixture was then grown in a culture medium at 37°C for 72 hours (3 days), after which the number of colonies was counted. The comparison of the number of bacteria before and after gargling showed that the number of bacteria decreased after rinsing with water. The average difference in the PHP index ($p<0.05$) before and after rinsing with water was 2. 25 and 1.94. From this study, it was found that there was no significant difference in the number of bacteria before and after rinsing with water.(26)

Research conducted by Titi Lasmini in 2020 used the Spread plate method of bacterial isolation. This research was conducted by isolating bacteria on MSA and MacConkey medium, purification, and testing for

biochemical reactions. The bacteria found in the oral cavity after gargling were bacteria of the *Staphylococcaceae* group (*Staphylococcus aureus* 3.85%, *Coagulase Negative Staphylococcus* 30.77%), *Streptococcaceae* (*Enterococcus* sp. 3.85%), HACEK Group (*Aggregatibacter* sp. 3.85%), *Enterobacteriaceae* (*Klebsiella* sp. 19.23%, *Citrobacter* sp. 3.85%), and *Nonfermenting Gram-Negative Bacilli* (*Acinetobacter* sp. 7.69%, *Pseudomonas* sp. 26.92%). The results showed that *Coagulase Negative Staphylococcus* (30.77%), *Pseudomonas* sp. (26.92%), and *Klebsiella* sp. (19.23%) were the most common species in the oral cavity of non-smokers.[27]

Another study conducted by Utami and Suryani in 2016 found the decrease in the number of germs was shown by counting the number of germs. The type of bacteria found in the mouth was *Staphylococcus* sp. with the number of colonies were 277 ± 178 CFU/ml before ablution and 270 ± 240 CFU/ml after ablution. The *Streptococcus* sp. colonies were also found in the mouth around 116 ± 95 CFU/ml before ablution and 83 ± 82 CFU/ml after ablution. The results of the Wilcoxon statistical analysis showed that the *p*-value for *Streptococcus* sp. in the mouth was p=0.30 (p>0.05) and *Staphylococcus* sp. was 0.002 (p<0.05). This result shows that there is a decrease in the number of germs before and after ablution against *Staphylococcus* sp. and *Streptococcus* sp. in the nurse's mouth.[28]

In addition, research conducted by Safitri K, H. L. in 2019 shows that a variety of bacterias, such as *Escherichia* sp. 40%, *Pseudomonas* sp. 33.33%, *Lactobacillus* sp. 6.67%, *Streptococcus* sp. 6.67%, *Fusobacterium* sp. 6.67%, *Veillonella* sp. 6.67% and the bacteria found after ablution was *Escherichia* sp. 13.33%, *Pseudomonas* sp. 26.67%, *Lactobacillus* sp. 20%, *Streptococcus* sp. 13.33%, and *Veillonella* sp. 26.67%. And also shows that there is an increase in gram-positive bacteria which is a normal flora in the mouth after ablution.[10]

A study conducted by Manurung in 2020, about the effectiveness of gargling steeped green tea (*Camellia sinensis*) on the number of bacterial colonies as an asepsis measure, found that the average number of colonies in the group after gargling with green tea was 90.83 ± 38.73 CFU/ml and the number of colonies in the mineral water gargling group was 218.55 ± 120.86 CFU/ml.[29]

In addition, a study conducted by Febriyanti in 2018 aimed to compare the number of anaerobic bacterial colonies in the saliva of children who were rinsed with peatland water and PDAM water. In the saliva of children who were rinsed with PDAM water, colonies of *Streptococcus* sp. and *Lactobacillus* sp. were found. In this study, the number of anaerobic bacterial colonies in peatland water was 217 CFU/ml, while the number of anaerobic bacteria colonies in PDAM water was 133 CFU/ml. Based on the results of the independent t-test (0.000) (p<0.05) showed that there was a significant difference between the number of anaerobic bacteria colonies that were rinsed with peatland water and PDAM water. This means that the number of anaerobic bacteria colonies in the saliva of children who rinsed with peatland water was higher than the number of anaerobic bacteria colonies in the saliva of children who rinsed with PDAM water.[30]

And research conducted by Mahgaidren in 2018 discussed the effectiveness of gargling a 15% forest honey solution and 1% povidone-iodine solution to reduce the number of oral bacteria in students of the Faculty of Dentistry, University of North Sumatra. In this study, aqua was used as a negative control where the average number of bacteria before treatment (pretest) was 188.43 × 103 ± 57.665 × 103 CFU/ml and after treatment (posttest) was 128.14 × 103 ± 37.791 × 103 CFU/ml. from these results indicate that there is a decrease in the number of bacteria in the oral cavity before and after gargling aqua.(31)

From some of these studies, the results showed that by carrying out ablution activities specifically meant in this case was gargling, it was found that there was a decrease in the number of bacteria in the mouth after gargling. Based on the description above, the bacteria found in the mouth before and during ablution can be classified into two types, namely gram-positive bacteria and gram-negative bacteria. The number of gram-negative bacteria tends to decrease after ablution, while the number of gram-positive bacteria increases. This happens because gram-positive bacteria are normal flora in the oral cavity that has an important role in the body's defense. By gargling, you can clean the oral cavity from bacteria and microbes before they spread and cause disease, and prevent the potential for dental and oral diseases. This proves that ablution can minimize the occurrence of problems in dental and oral health.

Conclusion

From the results of the analysis of a systematic study, it can be concluded that the types of bacteria present in the oral cavity before ablution are 33.33% *Pseudomonas* sp., 6.67% *Lactobacillus* sp., 3.33% *Streptococcus* sp. and 0.14% *Staphylococcus* sp. in the oral cavity after ablution were 26.8% *Pseudomonas* sp., 20% *Lactobacillus* sp., 5% *Streptococcus* sp. and 2% *Staphylococcus* sp. this indicates that there is an increase in gram-positive bacteria in the oral cavity after ablution.

Conflict of Interest

No potential conflict of interest relevant to this article was reported

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References

1. Kusumanto H. The Relationship between Periodontal Health Status and Learning Achievement in Students aged 10-12 Years at Mim Klaseman Gatak Sukoharjo. 2016;53(9):1689–99.
2. Indonesian Ministry of Health. Basic Health Research (RIKESDAS). Jakarta: Indonesian Ministry of Health Research and Development Agency; 2018. Available from: http://www.yankes.kemkes.go.id/assets/downloads/PMK No. 57 of 2013 concerning PTRM.pdf
3. Ren W, Zhang Q, Liu X, Zheng S, Ma L, Chen F, et al. Exploring the oral microflora of preschool children. J Microbiol. 2017;55(7):531–7.
4. Elkhaira R, Kasuma N, Son AE. Number of Lactic Acid Bacterial Colonies in a Healthy Oral Cavity. J Health Andalas. 2020;8(4):157–61.
5. Jawetz E, Melnick JL AE. Medical Microbiology. 27th ed. Jakarta: EGC; 2017. 199 p.
6. Chaturvedi M, Punj A. Human Oral Microflora. Int J Curr Adv Res [Internet]. 2017;7(7): 14065–70. Available from: http://dx.doi.org/10.24327/ijcar.2018.14070.2539
7. Maawiyah A. Thaharah as the Key of Worship. Sarwah J Islam Civiliz Thought. 2016;15(2).
8. Ajib M. Fiqh of Wudhu Version of the Syafi'i School [Internet]. South Jakarta: Fiqh Publishing House; 2019. 1–38 p. Available from: http://repo.iainbatusangkar.ac.id/xmlui/bitstream/handle/123456789/12275/1569222648762_Fiqih Wudhu Version of Madzhab Syafiiy.pdf?sequence=1
9. Afif M, Khasanah U. The Urgency of Wudhu and Its Relevance for Health (Study of Ma'anil Hadith) in the Perspective of Imam Musbikin. Riwayah J Study Hadith. 2019;3(2):215.
10. Safitri K HL. Isolation, Identification and Sensitivity Test of Antibiotic Germs in the Mouth Before and After Wudhu on Students of the 2016 Faculty of Medicine, Indonesian Muslim University. Indonesian Muslim Univ Makassar. 2019;
11. Ansory I. Wudu' Rasulullah SAW According to 4 Schools. South Jakarta: Fiqh Publishing House; 2018. 1–77 p.
12. Zulfa FF. The Benefits of Wudu on Health from the Perspective of the Hadith of the Prophet SAW. (A Study of Tahlili Hadith). State Islamic University of Alauddin Makassar. 2019;
13. Lumowa SVT. Bacteriology. East Java: RADe.Rozarie; 2016.
14. Padoli. Nursing Microbiology and Parasitology. Ministry of Health of the Republic of Indonesia: Agency for the Development and Empowerment of Health Human Resources; 2016.
15. MH's daughter, Sukini, Yodong. Dental Nursing Teaching Materials. Microbiology. Ministry of HealthRepublic of Indonesia: Agency for Health Human Resources Development and Empowerment; 2017.
16. Hegde MN. Dysbiosis of Oral Microflora: A Review. J Heal Allied Science NU. 2018;08(04):034–9.
17. Samaranayake L, Matsubara VH. Normal Oral Flora and the Oral Ecosystem. Dent Clin North Am. 2017;61(2):199–215.
18. Adnyani NP, Made I, Artawa B. Effect of Dental and Oral Diseases on Halitosis. J Dental Health. 2016;4(1):24–8.
19. Cruz Quintana SM, Diaz Sjostrom P, Arias Socarrás D, Mazón Baldeón GM. Microbiota of oral cavity ecosystems. Rev Cubana Estomatol. 2017;54(1):84–99.
20. Puspitasari AM, Ratnawati DE, Widodo AW. Classification of Dental and Oral Diseases Using the Support Vector Machine Method. J Developer of Information Technology and Computing Science. 2018;2(2):802–10.
21. Andriyani, Permatasari TAE, Purnamawati D, Putri A, Maududi AA Al. APPLYING CLEAN AND HEALTHY BEHAVIOR BY WUDHU FOR HEALTH BENEFITS. Indonesia J Islam Public Heal. 2021;1(1):1–7.
22. Shankhdhar K. Diabetic foot amputation prevention: wudu could be a boon in disguise. Diabetes Foot J. 2021;24(1):26–9.
23. Armiati IGK. Reduction in the number of Streptococcus mutans colonies in the oral cavity by ethanol extract of aloe vera leaf bark. Interdental J Dentistry. 2018;14(1):1–4.
24. Ernawati KL. KOMBUCHA TEA REDUCE THE NUMBER OF STREptococcus BACTERIA. Faculty of Dentistry, Mahasaraswati Univ, Denpasar. 2018;6–10.
25. Djohari M, Lestari R, Hasti S. Identification and test of the inhibitory activity of ethanol extract of areca nut (Areca catechu L.) against gum bacteria isolates. J Researcher Farm Indonesia. 2019;7(2):61–9.
26. Da-Young Kwak, Na-Yeon Kim, Hye-Jin Kim, Seung-Yeon Yang, Ji-Eun Yoon I-AH and S-HN. Changes in the oral environment after tooth brushing and oral gargling. Biomed Res. 2019;28(6):1–5.
27. Lasmini T. Identification of Mouth Cavity Bacteria of Smokers and Non-Smokers in Pekanbaru. J Anal Health Clinic Science [Internet]. 2018;8(1):17–27. Available from:

http://jurnal.univrab.ac.id/index.php/klinikal/article/view/525/361
28. Utami VHB, Suryani L. The Effectiveness of the Application of Wudhu in Reducing Germs Numbers on the Hands, Mouth and Nose of Nurses. Med Pearl. 2016;13(1):43–8.
29. Manurung DM. The Effectiveness of Gargling with Green Tea (Camellia Sinensis) on the Number of Bacterial Colonies as Asepsis in the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of North Sumatra. University of North Sumatra, Medan. 2020;
30. Febriyanti E, Kania TP D, Aspriyanto D. Comparison of Anaerobic Bacterial Colony Numbers in Children's Saliva Gargling with Peatland Water and PDAM Water. Dentist. 2018;II(1):113–7.
31. Mahgaidren R. The Effectiveness of Gargling with 15% Forest Honey Solution and 1% Povidone Iodine Solution on Reducing the Number of Bacteria in the Oral Cavity in Faculty Students. 2018; Available from: http://repositori.usu.ac.id/handle/123456789/5178

REVIEW ARTICLE **Open Access**

Antibacterial Potency of Black Garlic Extract from *Allium sativum* on *Escherichia coli*

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ABSTRACT

Background: Diarrhea is still one of the global issues especially in developing countries. Diarrhea can be caused by either an infectious agent or a non-infectious agent. *Escherichia coli* is one of the infectious agents that is responsible for causing diarrhea. Garlic (*Allium sativum*) is a plant that has a good antibacterial activity potential because of its organosulfur and phenolic compound. Black garlic is the product of spontaneous fermentation of garlic that has improved its bioactive compounds.
Content: Antibacterial potency of black garlic extract on *Escherichia coli* is shown on the resistance zone that formed where the lowest zone diameter is 9,67 mm while the highest zone is 24 mm. The fermentation of black garlic happened at the temperature of 70-80°C with 75-80% for 28-40 days.
Conclusion: Black garlic is shown to exhibit antibacterial activity against bacteria such as *Escherichia coli*. This fermented product has the potential to be a therapeutic agent for diseases caused by *Escherichia coli*.

Keywords: Garlic; Antibacterial; *Escherichia coli*

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Introduction

Diarrhea is still one of the major global issues with high morbidity and mortality rate in some countries, especially in developing ones (Hartati et al, 2018). According to data from World Health Organization (WHO) in 2013, every year there are approximately 1,7 billion cases of diarrhea with 760.000 mortality numbers on children under the age of 5. (1)

In Indonesia, the incidence of diarrhea for all ages according to Rapid Survey in 2019 is estimated at 270/1.000 morbidity rate and 843/1.000 for children. The total finding of diarrhea in medical facilities is 7.265.013 and 3.979.790 of them are children (Kemenkes RI, 2020). (2)

Based on the profile of Sulawesi Selatan's public health office per 2017, there is 257.108 citizen with diarrhea where 44.914 cases are from Makassar (Dinkes Sulsel, 2018). That data shows that diarrhea is still a common problem in current society. (3)

Diarrhea can be caused by an infectious agent and a non-infectious agent. The most common non-infectious agent is food allergies. Infectious agents, on the other hand, are caused by bacteria, viruses, or parasites. *Escherichia coli* (*E. coli)* is the most common pathogen group that causes diarrhea. Two among them are ETEC (*Enterotoxigenic Escherichia coli*) and EHEC (*Enterohemmorahic Escherichia coli*). ETEC is the most common cause of diarrhea in children in developing country while EHEC causes diarrhea from contaminated meat (Leon T, 2020).(4)

Garlic (*Allium sativum*) is one of the plants that have many medicinal properties, among them are antimicrobial. Its contents such as Allicin are useful to inhibit gram-positive and gram-negative bacteria by inhibiting the production of RNA and lipid synthesis, so the bacteria cannot thrive. The antibacterial capacity has more potential in gram-positive bacteria compared to gram-negative bacteria (Moulia MN et al, 2018). (5)

Garlic (*Allium sativum*) is also rich in chemicals that are useful as antioxidants. Organosulfur compounds such as allicin, adenosine, ajoene, flavonoid, and saponin provide many benefits, especially in the health sector (Wibisono Y et al, 2020). (6)

The results of the study prove that garlic extract (Allium sativum) has antibacterial potential against gram-positive bacteria (*Staphylococcus epidermidis, Streptococcus viridans*) and gram-negative bacteria (*Escherichia coli, Salmonella typhi*). However, the extract has a greater inhibitory effect on gram-positive than gram-negative bacteria (Airaodion AI et al, 2020) (7)

Black Garlic is the result of fermenting Garlic (*Allium sativum*) over a certain period with a high temperature (60-90ºC) and a high humidity (80-90%) environment. Its physicochemical changes increase the bioactivity of its components. Garlic's rich allicin content is converted into antioxidant components such as alkaloids and flavonoids. Allicin which gives a lot of flavor to garlic is broken down into other components such as diallyl sulfide, diallyl disulfide, and ajoene (Kimura S et al, 2017).(8)

The description above encourages the researcher to find out more about the potential of black garlic in increasing the effectiveness of garlic as an antibacterial product, especially on gram negative-bacteria. The results of this study are expected to prove the effect of black garlic extract as a more efficient antimicrobial to inhibit the work of *Escherichia coli* bacteria.

Content

This research uses the literature review method with a narrative review design. Journals that are taken as data are from both international and national journals released from the year 2017-2021 that are considered relevant to the topics.

The inclusion criteria are International Journal, National Journal, or textbook with keywords consisting: Antibacterial activity of black garlic on *Escherichia coli*, Disc diffusion method of black garlic on *Escherichia coli*, Black garlic effect on *Escherichia coli*, from the year 2017-2021. Electronic-based media includes sources from Elsevier / Clinical Key, PubMed and, Google Scholar.

While the exclusion criteria are journals that are found not in the form of full text (can't be fully accessed) and journals that are not from the web with international or national standards.

From keyword searches, obtained results as many as 203 journals. All journals are included in the search and then filtered. In the end, 8 remaining journals matched the inclusion criteria, which will then be used for this literature review.

Of the 8 journals, 6 journals were research conducted using a true experimental design method to see the antibacterial effect of black garlic on *Escherichia coli*. In the research of Thalia et al (2020) the largest inhibition zone was 12.8 mm with black garlic fermented at 80°C for 40 days.[9] From research done by Setiyoningrum et al (2021) obtained the largest inhibition zone of 24 mm with ethyl acetate solvent and 18.9 mm with isopropanol solvent using black garlic which was fermented at a temperature of 72°C for 4 weeks.[10] In the research of Chang et al (2021) the largest inhibition zone was 9,67 mm with Michiu Tou *wine* as a solvent on black garlic that was fermented at 70°C for 35 days.[11] Astuti et al (2018) used black garlic's essential oil with the largest inhibition zone at 13,16 mm.[12] Kang et al (2017) got 11,65 mm for its largest inhibition zone using black garlic that sold in the market.[13] Sasaki et al (2017) also proved that black garlic fermented at 70°C for 30 days gives an antibacterial effect on *Escherichia coli*.[14]

While the other 2 journals use the literature review method. In the research of Botas et al (2019), it was found that the antibacterial test of black garlic showed sensitive results to *Escherichia coli* 0157:H7.[15] In Ahmed et al (2021) research it was found that black garlic, after going through pre-clinical and clinical studies, showed clear evidence that its consumption can be a supportive therapy for various diseases in humans.[16]

Based on 8 journals that have been researched, it was found that in general black garlic extract has an antibacterial effect against *Escherichia coli*. The fermentation process carried out by each study to obtain black garlic extract also varied. Of the 8 journals, 4 journals did not explain the fermentation of black garlic samples, while in the other 4 journals, various black garlic fermentation processes were found. In a study conducted by Thalia et al (2020) the most effective fermentation is at the temperature of 80°C with an oven time of 40 days. Research by Setiyoningrum et al (2021) said that 4-week fermentation with the temperature of 72°C and 80% humidity showed the best antibacterial activity. In a journal by Chang et al (2021) it was stated that the most optimal black garlic fermentation is for 35 days at a temperature of 70°C with 85% humidity. Meanwhile, in the study of Sasaki et al (2017) black garlic extract was fermented at a temperature of 70°C with 75% humidity for 30 days.

Based on those 4 studies, it can be seen that the average fermentation is done at the temperature of 70-80°C with 75-85% humidity for 28-40 days. This is following the journal *Novel Nutritive Garlic Product "'Black Garlic'": A Critical Review of Its Composition, Production, and Bioactivity* by Alihanoğlu S et al (2017) who said that fermentation to produce the best characteristic black garlic is done at high temperatures (70-80°C) with 70-90% humidity for about 21 days.[17]

In a study conducted by Chang et al (2021) it was said that fermented garlic had more bacteriosatic capacity due to the presence of antibacterial agents besides allicin in black garlic. This is following the journal *Converting organosulfur compounds to inorganic polysulfides against resistant bacterial infections* by Xu Z et al (2018) which stated that the bacteriostatic capacity of garlic was mostly obtained from Allicin derivates, such as diallyl trisulfide (DATS), diallyl disulfide (DADS), diallyl sulfide (DAS).[18] And in accordance to journal *Biological Activities of Black Garlic Fermented with Lactobacillus Plantarum PN05 and Some Kinds of Black Garlic Presenting Inside Vietnam* by Ngan N et al (2017) this component increased by five to six times in the form of a more stable organosulfur compound namely SAC.[19]

The antibacterial power of black garlic extract itself is obtained from the constituent components of black garlic which are rich in organosulfur and phenolic compounds. The organosulfur component that increased dramatically due to the fermentation process is a compound named S-allylcysteine (SAC), which plays a major role in antioxidant activity to ward off free radicals. The content of phenolic bioactives such as polyphenols and flavonoids also increases due to the heating effect during fermentation. Antimicrobial activity in black garlic is not only obtained from the allicin content which is still present in black garlic but can also come from the content of phenolic compounds by inhibiting RNA synthesis quickly and thoroughly and partially DNA and protein synthesis. This damages the cell membrane resulting in inhibition of the activity and biosynthesis of specific enzymes needed in bacterial metabolic reactions

In the journal *Influence of thermal processing on the bioactive, antioxidant, and physicochemical*

properties of conventional and organic agriculture black garlic (Allium sativum l.) by Najman k et al (2020) it is known that *S-allylcysteine* (SAC) is the most important bioactive compound that plays a major role in black garlic's pharmacological effects.[20] *S-allylcysteine* (SAC) is a component that exhibits very high antioxidant activity. This is following the research of Thalia et al (2020) which showed that the antioxidant activity and total phenolic compound of black garlic were twice those of unfermented garlic.

In the research of Botas J et al (2019) it was found that black garlic had antibacterial activity on all bacterial isolates it was tested on. The study showed that black garlic produced bactericidal activity although the amount was not significant. Minimal bactericidal concentration (MBC) was found to be most visible in *Escherichia coli* and *Staphylococcus aureus*. This incident can be related to the flavonoid content in black garlic. In the journal *Efektivitas Daya Hambat Bakteri Ekstrak Bawang Dayak Terstandarisasi Flavonoid Terhadap Enterococcus Faecalis (In Vitro)* by Armanda F et al (2017) it is said that flavonoids are phenolic compounds that have a toxic effect on bacteria due to their ability to damage the hydrogen bridge bonds of double-stranded DNA strands. Flavonoid compounds will contact the DNA in the cell nucleus and through the difference in polarity between the lipids that make up DNA and the alcohol groups on the flavonoid compounds a reaction will occur, thereby damaging the lipid structure of the DNA and eventually the bacterial cell nucleus will also lyse and die.[21]

Research conducted by Ahmed T (2021) showed the results of giving black garlic in-vivo to various types of rats. These studies show a positive therapeutic effect against various diseases. This is following with the journal *Black Garlic and Its Therapeutic Benefits. In: Medicinal Plants - Use in Prevention and Treatment of Diseases* by Tran G (2020) says that consumption of black garlic can be a supportive therapy for diseases because of its anticancer, antiobesity, antioxidant, and anti-inflammatory effects.[22]

Conclusion

From the results and discussions, it can be concluded that black garlic extract has an antibacterial effect against *Escherichia coli*. The inhibition zone formed after giving black garlic extract averaged at 9,67 mm – 24 mm. The average black garlic fermentation is carried out at a temperature of 70-80°C and 75-80% humidity for 28-40 days. The greatest bacterial inhibition was found in 4 weeks of fermentation with a temperature of 72°C and 80% humidity with *Ethyl acetate* dan *Isopropanol* solvents. Black garlic fermentation increases the antioxidant activity and total phenolic compounds of garlic (*Allium sativum* L). Organosulfur compounds such as S-allylcysteine (SAC) and flavonoid compounds are the active substances in black garlic that play the most role in inhibiting *Escherichia coli.*

Suggestion for further research, research should be carried out to study the different use of different solvents while making black garlic about the antibacterial effects of said black garlic on *Escherichia coli*. In

addition, it is necessary to study the effect of black garlic extract on other bacteria. The purpose is to see the spectrum of antibacterial effects of black garlic.

Conflict of Interest

No potential conflict of interest relevant to this article was reported

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References

1. Hartati S, Nurazila N. Faktor Yang Mempengaruhi Kejadian Diare Pada Balita Di Wilayah Kerja Puskesmas Rejosari Pekanbaru. J Endur. 2018;3(2):400.
2. Kementerian Kesehatan Republik Indonesia. Data dan Informasi kesehatan indonesia 2019. Profil Kesehat Indones. 2020;8(9):1–213.
3. Selatan DKPS. Profile Kesehatan Provinsi Sulawesi Selatan. J Chem Inf Model. 2018;53(9):1689–99.
4. Leon T De. IV Digestive System [Internet]. Conn's Current Therapy 2020. Elsevier; 2020. 174–177 p. Available from: http://dx.doi.org/10.1016/B978-0-323-71184-5.00042-2
5. Moulia MN, Syarief R, Iriani ES, Kusumaningrum HD, Suyatma NE. Antimikroba Ekstrak Bawang Putih. J Pangan. 2018;27(1):55–66.
6. Wibisono Y, Izza N, Savitri D, Rosalia Dewi S, Wahyu Putranto A. Ekstraksi Senyawa Fenolik Dari Bawang Putih (Allium sativum L.) Untuk Agen Anti-Biofouling Pada Membran. J Ilm Rekayasa Pertan dan Biosist. 2020;8(1):100–9.
7. Airaodion AI, Ngwogu AC, Ngwogu KO, Ekenjoku JA, Megwas AU. Pharmacotherapeutic Activity of Allium sativum (Garlic) Bulb against Gram-positive and Gram-negative Bacteria. Asian J Res Infect Dis. 2020;3(3):22–7.
8. Kimura S, Tung YC, Pan MH, Su NW, Lai YJ, Cheng KC. Black garlic: A critical review of its production, bioactivity, and application. J Food Drug Anal [Internet]. 2017;25(1):62–70. Available from: http://dx.doi.org/10.1016/j.jfda.2016.11.003
9. Thalia CU, Chrisnasari R, Rosita Dewi AD. Pengaruh Pengolahan Terhadap Nilai Fungsional Bawang Putih (Allium sativum). KELUWIH J Sains dan Teknol. 2020;1(1):1–14.
10. Setiyoningrum F, Herlina N, Afiati F, Priadi G. Antibacterial activities of Solo garlic. IOP Conf Ser Mater Sci Eng. 2021;1011:012067.
11. Chang TC, Jang H Der. Optimization of aging time for improved antioxidant activity and bacteriostatic capacity of fresh and black garlic. Appl Sci. 2021;11(5):1–16.
12. Astuti DP, Palupi C. Perbandingan Efektivitas Antibakteri Minyak Atsiri Bawang Putih (Allium sativum) Dan Black Garlic Terhadap Bakteri Staphylococcus Aureus Dan Escherichia coli Dengan Metode Kirby-Bauer. J Pharm Sci Med Res. 2018;1(2):17.
13. Kang JH, Son HJ, Min SC, Oh DH, Song K Bin. Antimicrobial activity of black garlic pomace extract and its application to cleansing of fresh spinach leaves for microbial control. J Korean Soc Food Sci Nutr. 2017;46(4):450–8.
14. Méndez-Vilas A. Science within food up-to-date advances on research and educational ideas. 2017.
15. Botas J, Fernandes Â, Barros L, Jos M, Carvalho AM, Ferreira ICFR. A Comparative Study of Black and White Allium sativum L : Nutritional Composition and Bioactive Properties. Mdpi. 2019;24:2194.
16. Ahmed T, Wang C. Black Garlic and Its Bioactive Compounds on Human Health Diseases: A Review. Molecules [Internet]. 2021 Aug 19;26(16):5028. Available from: https://doi.org/10.3390/molecules26165028

17. Alihanoğlu S, Karaaslan M, Vardin H. Novel Nutritive Garlic Product "'Black Garlic'": A Critical Review of Its Composition, Production and Bioactivity. Harran Üniversitesi Mühendislik Derg. 2017;2(3):57–63.
18. Xu Z, Qiu Z, Liu Q, Huang Y, Li D, Shen X, et al. Converting organosulfur compounds to inorganic polysulfides against resistant bacterial infections. Nat Commun [Internet]. 2018;9(1):1–13. Available from: http://dx.doi.org/10.1038/s41467-018-06164-7
19. Ngan N, Giang M, Tu N. Biological Activities of Black Garlic Fermented with Lactobacillus plantarum PN05 and Some Kinds of Black Garlic Presenting Inside Vietnam. Indian J Pharm Educ Res. 2017;51(4):672–8.
20. Najman K, Sadowska A, Hallmann E. Influence of thermal processing on the bioactive, antioxidant, and physicochemical properties of conventional and organic agriculture black garlic (Allium sativum l.). Appl Sci. 2020;10(23):1–17.
21. Armanda F, N MYI, Budiarty LY. Efektivitas Daya Hambat Bakteri Ekstrak Bawang Dayak Terstandarisasi Flavonoid Terhadap Enterococcus Faecalis (In Vitro). Dentino. 2017;2(2):183–7.
22. Tran G-B, Pham T-V, Trinh N-N. Black Garlic and Its Therapeutic Benefits. In: Medicinal Plants - Use in Prevention and Treatment of Diseases [Internet]. IntechOpen; 2020. p. 13. Available from: https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics

ORIGINAL ARTICLE **Open Access**

Relationship of Spiritual Aspect with Hyperemesis gravidarum Incidence of Pregnant Women in Tinambung Disctrict in 2020

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ABSTRACT

Introduction: Nausea and vomiting is a common thing and physiological in early pregnancy. This condition can be severe if continued and causes a pregnancy disorder called *hyperemesis gravidarum*. Mothers with *hyperemesis gravidarum* based on data at the Tinambung Health Center in 2018 amounted to 47 people and in 2019 there were 58 people. The risk factor for *hyperemesis gravidarum* is associated with several factors, one of which is the spiritual aspect of the mother.
Methods: This type of research is an analytic survey using cross-sectional. Sampling from 344 populations with a purposive technique for 78 samples. Data analysis using Chi-Square test on SPSS program.
Results: The Majority of respondents had moderate and low spiritual aspects, namely 33 respondents (42.3%). The chi square test showed p-value = 0.042 (<0.05) so that the spiritual aspect significantly affected the status of hyperemesis gravidarum.
Conclusion: This study concludes that there is a significant relationship between the spiritual aspects of the mother with the occurrence of hyperemesis gravidarum in Tinambung District.

Keywords: Nausea Vomiting*; Hyperemesis gravidarum;* Risk Factors*;* Mother's Spiritual Aspect

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Introduction

Nausea and vomiting are common thing and physiological in early pregnancy (1–3). The Ministry of Health explained that more than 80% of pregnant women experience nausea and vomiting which can cause women to avoid certain foods and increase the risk to the mother and her fetus. (4) This condition can be severe if it persists and causes a pregnancy disorder called *hyperemesis gravidarum*.

Hyperemesis gravidarum is the most common complication in the first trimester of pregnancy. (4) This situation can be bad and interfere with daily activities until dehydration occurs. (1) Although hyperemesis gravidarum rarely causes death, its incidence is still very common in the community. 25% of patients with hyperemesis gravidarum are hospitalized more than once during pregnancy and sometimes mothers who experience this condition get depressed and even make them feel like terminating their pregnancy in extreme cases.(4)

The case of hyperemesis gravidarum is influenced by several risk factors, one of them being the spiritual aspect of the mother. Spirituality of pregnant women is a belief that comes from within pregnant women that connects them to the Creator or Allah SWT, themselves, and the surrounding environment. This factor is associated with the level of anxiety in pregnant women which can trigger excessive stress so that the mother experiences excessive nausea and vomiting as well (5)

The case of hyperemesis gravidarum based on data from the World Health Organization (WHO) reaches 12.5% of all pregnancies in the world. Meanwhile, for the incidence of hyperemesis gravidarum in Indonesia, based on research by Indrayani (2018), there are 14.8% of all pregnancies (6). Based on data from the West Sulawesi Provincial Health Office, the number of pregnancies complicated by hyperemesis gravidarum is 310 people (7). Pregnant women with *hyperemesis gravidarum* in Tinambung District, Polewali Mandar regency, West Sulawesi province based on data from the Tinambung Health Center in 2018 amounted to 47 people and in 2019 as many as 58 people (5).

Islam has also explained the condition of the mother during pregnancy to caring for the child, Allah SWT says in Q.S Luqman/31:14:

وَوَصَّيْنَا الْاِنْسَانَ بِوَالِدَيْهِۚ حَمَلَتْهُ اُمُّهٗ وَهْنًا عَلٰى وَهْنٍ وَّفِصٰلُهٗ فِيْ عَامَيْنِ اَنِ اشْكُرْ لِيْ وَلِوَالِدَيْكَۗ اِلَيَّ الْمَصِيْرُ ١٤

Translation:
“And We command man (to do good) to his parents. His mother had conceived him in a state of increasing weakness and weaned him at the age of two years. Be grateful to me and both parents. Only to Me will you return”. (9)

Based on the data above, it can be seen that there has been an increase in the case of hyperemesis gravidarum in Tinambung District in the last two years and it is known that Tinambung District is one of the areas with high spiritual values, so the authors are interested in research to determine the relationship

between mother's spiritual aspects with the incidence of pregnant women with hyperemesis gravidarum in Tinambung District in 2020.

Methods

This research is an analytical survey research with a cross sectional approach and was carried out in Tinambung District, Polewali Mandar Regency, and West Sulawesi Province. The population in this study were 344 pregnant women who performed Antenatal Care (ANC) in the working area of the Tinambung Health Center in 2020 with a sampling technique, namely purposive sampling by setting certain criteria, namely pregnant women who performed Antenatal Care (ANC) at the Tinambung Health Center in 2020, pregnant Muslim, and willing to be a respondent so that the number of samples obtained is 78 respondents.

The data collection in this study used secondary data in the form of patient medical records in the work area of the Tinambung Health Center in 2020 and primary data through filling out questionnaires and direct interviews with respondents. The data analysis method in this study used the Statistical Package for The Social Sciences (SPSS) computer program with univariate data analysis to describe each research variable and bivariate analysis to analyze the relationship between the dependent and independent variables. Data analysis used the chi square test in the X2 test to test the independence between the two variables arranged in the row and column table with = 0.05, meaning that the null hypothesis (H0) is rejected if p value <α which means there is a relationship between the dependent variable and the independent variable.

Result

Table 1
Frequency Distribution of Respondents Based on Mother's Spiritual Aspect In Tinambung District, Polewali Mandar Regency

Mother's Spiritual Aspect Level	Frequency	
	n	%
Very Low	7	9,0
Low	33	42,3
Moderate	33	42,3
High	5	6,4
Total	**78**	**100**

Primary Data, 2021

Comment [WU1]: Font 11, bold

Based on table 1, it is known the frequency of the independent variables used. The spiritual aspect variable is known that the majority of respondents have spiritual aspects in the medium and low categories, namely as many as 33 respondents or 42.3%.

Table 2
Frequency Distribution of Respondents Based on Hyperemesis gravidarum Status in Pregnant Women in Tinambung District, Polewali Mandar Regency

Hiperemesis gravidarum Status	Frequency	
	n	%
Emesis Gravidarum	33	42,3
Mild Hiperemesis	45	57,7

gravidarum		
Total	**78**	**100**

Primary Data, 2021

Based on table 2, it is known that the majority of respondents have mild hyperemesis gravidarum status as many as 45 respondents or 57.7%. For Emesis gravidarum status as many as 33 respondents or 42.3%.

Tabel 3
Relationship Between Mother's Spiritual Aspect and Hyperemesis gravidarum in Pregnant Women in Tinambung District, Polewali Mandar Regency

Mother's Spiritual Aspect	Hiperemesis Status		P Value
	Emesis Gravidarum	*Hiperemesis gravidarum* Mild	
Very Low	4	3	0,042*
Low	8	25	
Moderate	19	14	
High	2	3	
Total	**33**	**45**	

Primary Data, 2021

Based on table 3, we can know the relationship of the independent variable given to the hyperemesis status variable as the dependent variable. The spiritual aspect variable is known that the majority of respondents have low spiritual aspects with mild hyperemesis gravidarum status, namely as many as 25 respondents.

The results of the chi square test indicate that the spiritual aspect variable has a significant effect on the status of hyperemesis gravidarum. It is known that the p value for this variable is less than 0.05, which means reject H0 so that there is a significant relationship between the spiritual aspect variable and the status of hyperemesis gravidarum.

Discussion

Most respondents who experienced hyperemesis gravidarum were at a low level of spiritual adaptation. This is following with research conducted by Miladina Nahar (2018). Spearman Rank Correlation (rho) statistical test results show that there is a relationship between the spiritual aspect and the anxiety experienced by pregnant women (p value <0.05) with r value = -0.224. Where the higher the spiritual level obtained, the lower the level of anxiety experienced by pregnant women (7).

Anxiety can trigger stress in pregnant women which are considered as one of the factors causing hyperemesis gravidarum. Hyperemesis gravidarum is a manifestation of psychological disorders that are

changed in the form of physical symptoms. Pregnant women under stress conditions can experience an increase in blood pressure and heart rate so that they can increase the hormone Human Chorionic Gonadotropin (HCG) which can stimulate nausea and vomiting (3,11,12)

Mental health is closely related to religious beliefs. The spiritual aspect is an essential part of one's overall health and well-being (13) Spiritual adaptation in one's life will create a sense of love, faith, hope, and trust. In spiritual principles, the thought of a thing is usually something that ends up happening. Pregnant women who do not get the touch of the value of Godhead, do not form a relationship with Allah SWT, are far from Allah, and the condition of their soul is not able to be bound to Allah, then their soul will be weaker and dry. The need for serenity to feel comfortable during pregnancy is the basis for the importance of spiritual adaptation, especially during the pregnancy (11)

Spiritual adaptation has two forms of implementation, relationship with God (habluminallah) and relationship with humans (habluminannas). A pregnant woman needs a good relationship between the two. The relationship with God will be a source of strength that comes from oneself, while the relationship with humans will lead to other forces that come from outside (14). As research conducted by Price (2007) with the conclusion that spirituality can build and maintain the respondent's relationship with God, which is also able to have a significant positive impact on the health of the respondents. In addition, spiritual adaptation is also needed by pregnant women because spirituality can bring calm which has a positive impact on fetal development (15)

Conclusion

There is a significant relationship between the incidences of hyperemesis gravidarum with spiritual aspects in pregnant women. Special attention is needed from primary level health services to minimize risk factors for the occurrence of hyperemesis gravidarum in pregnant women and other research is needed on risk factors that were not examined in this study.

Conflict of Interest

None

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References

1. Nurnaningsih. Gambaran Faktor-faktor Kejadian Hiperemesis Gravidarum Pada Ibu Hamil Trimester Pertama Di RSKDIA Siti Fatimah Tahun 2012. 2012.

2. Rorrong JF, Wantania JJE, Lumentut AM. Hubungan Psikologis Ibu Hamil dengan Kejadian Hiperemesis Gravidarum. e-CliniC [Internet]. 2021; Available from: https://ejournal.unsrat.ac.id/index.php/eclinic

3. Fifi Ria Ningsih Safari. Hubungan Karakteristik dan Psikologis Ibu Hamil dengan Hiperemesis Gravidarum di RSUD H. ABD. Manan Simatupang Kisaran. 2017;

4. Oktavia L. Kejadian Hiperemisis Gravidarum Ditinjau dari Jarak Kehamilan dan Paritas. Jurnal Aisyah : Jurnal Ilmu Kesehatan. 2016;1(2):41–6.

5. Ayu Wijayanti Hutauruk. Asuhan Keperawatan Pada Klien yang Mengalami Hiperemesis Gravidarum dengan Kekurangan Volume Cairan di Rumah Sakit Umum Daerah Pandan Kabupaten Tapanuli Tengah Tahun 2020. 2020;

6. Indrayani T. Faktor-Faktor Yang Berhubungan Dengan Kejadian Hiperemesis Gravidarum Di Rsud Dr. Drajat Prawiranegara Kabupaten Serang Tahun 2017. Jurnal Akademi Keperawatan Husada Karya Jaya [Internet].2018;4(1):9–21.Availablefrom: http://ejurnal.husadakaryajaya.ac.id/index.php/JAKHKJ/article/view/70/63%0Ahttp://ejurnal.husadakaryajaya.ac.id/index.php/JAKHKJ/article/view/70

7. Dinas Kesehatan Provinsi Sulawesi Barat. Data Jumlah Ibu Hamil Hiperemesis Gravidarum. Data Jumlah Ibu Hamil Hiperemesis Gravidarum. 2019;

8. Puskesmas Tinambung. Data Sekunder Jumlah Ibu Hamil Hiperemesis Gravidarum. Data Sekunder Jumlah Ibu Hamil Hiperemesis Gravidarum. 2019;

9. Kementerian Agama Republik Indonesia. Al-quran dan Terjemahan. 2019.

10. Sulistyowati, Soesanto E, Purwanti I. Hubungan antara tingkat stres dengan kejadian hiperemesis gravidarum pada ibu hamil trimester i di BPS Ny. Sayidah Kendal. Jurnal Unimus [Internet]. 2014;14–8. Available from: http://jurnal.unimus.ac.id/index.php/jur_bid/article/viewFile/1074/1123

11. Martins A, Pinto S, Caldeira S, Pimentel F. Translation and adaptation of the Spirituality and Spiritual Care Rating Scale in portuguese palliative care nurses. Revista de Enfermagem Referência. 2015 Mar 29;IV Série(N^{o} 4):89–97.

12. Nurnaningsih. Gambaran Faktor Faktor Kejadian Hiperemesis Gravidarum Pada Ibu Hamil Trimester Pertama Di RSKDIA Siti Fatimah Tahun 2012. Vol. 66. 2012. 37–39.

13. Miladina Nahar. Hubungan Spiritual Support Dengan Kecemasan Dan Adaptasi Spiritual Ibu Hamil.

2018;

14. Ulfa NA. Pengaruh Pendampingan Spiritual Membaca Al-Qur'an terhadap Adaptasi Spiritual dan Kecemasan Ibu Hamil. 2017;

15. Price S, LM, BG, CG, QC, & CTO. The Spiritual Experience of High-Risk Pregnancy. JOGNN Clinical Research. 2007;36(1):63–70.