International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Hemoglobin and Ferritin Serum Levels on Leprosy Patients before Multi Drug Therapy - World Health Organization (Mdt - Who) Compared with Healthy Control Group

Heruni Amalia[a]*, Farida Tabri[b], Sri Vitayani Muchtar[c], Ilhamjaya Petellongi[d], Rizalinda Sjahril[e], Safruddin Amin[f]

[a,b,c,f]*Department of Dermatology and Venereology, Faculty of medicine, Hasanuddin University, Makassar, Indonesia*

[d]*Department of Biostatistic, Faculty of medicine, Hasanuddin University, Makassar, Indonesia*

[d]*Department of Physiology, Faculty of medicine, Hasanuddin University, Makassar, Indonesia*

[e]*Department of Medical Microbiolog, Faculty of medicine, Hasanuddin University, Makassar, Indonesia*

[a]*Email: heruniakbar@gmail.com*

Abstract

Leprosy is an infectious disease caused by bacteria Mycobacterium leprae. The research aimed at investigating the hemoglobin and ferritin serum levels on the leprosy patients compared with the healthy control group, whether the anemia occurred on the leprosy patients. The research was conducted in Dr. Wahidin Sudirohusodo Hospital and other hospitals in Makassar City from April to July 2017. The research used the analytic observational method with the case control study design. Samples were the leprosy patients and the control group of 18 - 52 years old who came to be treated to the dermatovenereology of Dr. Wahidin Sudirohusodo Hospital and other hospitals in Makassar City. As many as 37 samples consisted of 19 samples of the leprosy patients and 18 samples of the control group.

---

* Corresponding author.

Blood from the vein of mediana cubiti was taken, the hemoglobin examination was carried out using the hematology analyzer and the ferritin serum was conducted using ELFA technique to find out every level. The research result indicates that the mean hemoglobin level on the leprosy patients is 13.1H.9 gr/dl lower than the control group with 14.2±1.3 gr/dl, the ferritin serum level is higher on the leprosy patients with the mean of 236.9±204.9 than the control group with 132.9±85.4. From 37 samples, the lowest hemoglobin level is 9.5 gr/dl, and the highest is 16.3 gr/dl with the mean of 13.6±1.7 gr/dl, the lowest ferritin serum level is 6.5 pg/ml, and the highest is 692.5 pg/dl with the mean of 186.3±165.0 pg/dl.

Keywords: Leprosy; hemoglobin level; ferritin serum level; anemia.

1. Introduction

Leprosy or Hansen's disease, is a chronic granulomatous bacterial infection that primarily affects the peripheral nerves and may subsequently attack skin, oral mucosa, upper airway, reticuloendothelial system, eyes, muscles, bones and testes except the central nervous system [1].

The bacterial that causes leprosy is *Mycobacterium leprae* or Hansen's germ, which was first identified by the Norwegian physician, Gerhard HenrikArmauer Hansen in 1873.

Globally, there is an upward trend in both leprosy prevalence and in the detection of new leprosy cases in 2012 to mid-2013. In Indonesia, the number of new cases detected in 2011 until 2012 appears to have declined slightly, with 22.390 cases in 2011 and 18.994 cases in 2012. Based on this report, most cases (82.6%) are multibacillary leprosy (MB) [2].

Chronic infectious diseases such as leprosy can cause anemia on its course. The changes in the formation of erythrocytes (eritropoiesis) results in anemia due to inflammatory process or better known as anemia of chronic diseases. Clinical features of anemia of chronic disease,are often asymptomatic and obscured by clinical symptoms of its underlying disease, thus requiring further evaluation. Anemia due to chronic disease occurs in patients with acute or chronic immune activity, so-called anemia of inflammation.

Leprosy is an infectious disease caused by Mycobacterium leprae,an intracellular obligate bacteria. All bacteria require a purine base from the nucleotides in order to make nucleic acids and also for oxidative metabolism. Unlike other mycobacteria, M. leprae cannot synthesize and may obtain it from a host cell. Mycobacteria also requireiron that they extract and procure it from the host through chelation with mycobactin. Iron is indispensable in various metabolic processes such as oxygen transport, DNA synthesis and transport of electrons as essential nutrients. Iron is also imperative as an energy cofactor in mitochondrial respiration, proliferation and activation of T lymphocytes, B lymphocytes, and Natural Killer cells.

Intake of iron from host tissue plays an important role for the development of pathogenic bacteria in vivo. The limited amounts of iron in the host trigger bacteria to take iron reserves from transferrin and lactoferrin, or from ferritin. Mycobacteria produces an iron-binding molecule (siderofor) called mycobactin, exochelin, and carboxymycobactin, which then stores it in the form of bacterioferritin. Gram-positive mycobacteria tend to

acquire iron-bound hemerather than ferritin, in which heme is the largest iron-binding protein in the body (80%). Mycobacteria have the ability to prevent phagosome maturation, thus providing an appropriate environment for the bacteria to grow.

Reduced iron reserves will decrease cell mediated immuneresponse such as delayed hypersensitivity, lymphocyte proliferation due to antigen stimulation, natural killer cytotoxicity and others. The presence of iron at the cellular level is required in the differentiation and proliferation of Th-1 and Th-2. Th-1 is more sensitive to antitransferrin antibody receptor, with the end result inhibition of DNA synthesis. Th-2 is relatively more resistant to these antibodies. It is therefore suspected that Th-1-mediated function is more sensitive to iron hemostasis in the body. Ferritin is the main protein associated with iron deposits by macrophages and hepatocyte cells. In 1997, it was proven that MDT therapy in less than 24 months was effective, thus the recommended duration was 1 year for MB leprosy [3]. Until now, early detection and therapy with MDT is still a major strategy in reducing the burden of disease caused by leprosy. MDT also succeeds in shortening the duration of infectivity thereby reducing the risk of subsequent transmission to a healthy person in the community. Previous study by Dogra and his colleagues [4], conducted on 730 MB leprosy patients in northern India who were treated with MDT for 12 months showed clinical, bacteriology and histopathological improvement in almost all patients. As far as the author's knowledge, there has been no study that reported hemoglobin and serumferritin levels in leprosy patients prior to MDT-WHO therapy compared to healthy controls. Hemoglobin and ferritin serum levels prior to therapy of subjects who met the inclusion criteria based on WHO were measured, in which the results were monitored and assessed. Based on the above background, this study aims to determine the levels of hemoglobin and serum ferritin in leprosy patients compared to healthy control and the incidence of anemia in leprosy patients.

2. Material and Methods

Time and Place

2017. The study was conducted in theDermatovenereology Department of Dr.WahidinSudirohusodo Hospital, Hasanuddin University Hospital,TajuddinKhalid Hospital and other networking hospitals in Makassar as sampling places. The study was conducted in April 2017 to July

Design Study and Variable

The research used the analytic observational method with the case control study design. The study variables consisted of: independent variable (leprosy patients pre MDT-WHO), dependent variable (hemoglobin and ferritin serum levels), intermediate variable (*Mycobacteriumleprae*, iron, erythrocytes), and confounding variable (food intake).

Population and Sample

The population of this study is leprosy patients who have not received MDT-WHO therapy who came to Dermatovenereology Department of Dr.WahidinSudirohusodo Hospital, Hasanuddin University Hospital

Makassar, Tajuddin Khalid Hospital Makassar and other networking hospitals. The study sample was the entire affordable population that met the inclusion criteria.

Data Collection

Every subjects who came to Dermatovenereology department and met the inclusion criteria were recorded and then history taking and physical examination were performed to establish the diagnosis of leprosy and photos of the subject were taken. After the patient signs the informed consent, a blood specimen was collected to determine the hemoglobin levels using hematology analyzer and serum ferritin levels using ELFA technique. The data obtained then analyzed and reported in the results.

Data Analysis

The collected data was processed and presented in the form of tables and graphics. Data analysis was performed using SPSS version 22.0 system with Independent sample t test and Mann Whitney test with $p < 0.05$ significance.

3. Results

An analytic observational approach with case control study was conducted to determine hemoglobin and serum ferritin levels in leprosy patients compared to healthy control and to determine the incidence of anemia. The study was executed atdermatovenereology clinic of Dr.WahidinSudirohusodoHospital Makassar, Hasanuddin University Hospital Makassar, Tajuddin Khalid Hospital Makassar and other networking hospitals from April to July 2017. Total samples were 37 subjects, consisted of 19 new leprosy patients with no history of previous therapy and 18 healthy subjects as the control group. The subjects aged 18 - 52 years old with the mean age 29.4; 25 men and 12 women with Hb levels ranged from 9.5 to 16.3 gr% with mean of 13.6 gr / dl and serum ferritin level ranged from 6.5-692.5 gr / dL with mean of 186.3 gr / dL; the results of serum ferritin levels were variable (attachment, Table 1).

Table 1: Sampel Characteristics

Variabelis		N	%	Min/Maks	Mean ± SD
Age		37	100,0%	18/52	29,4±10,6
Sex	Male	25	67,6%	-	-
	Female	12	32,4%	-	-
Group	Leprosy	19	51,4%	-	-
	Control	18	48,6%	-	-
Hb level (gr/dl)		37	100,0%	9,5/16,3	13,6±1,7
Ferritin serum level (ng/ml)		37	100,0%	6,5/692,5	186,3±165,0

Anova One-Way analysis showed that there was no significant difference in serum ferritin levels based on age group. While,there was a significant difference (p <0.05) in hemoglobin level based on sex group; Hb levels were higher in males (14.3 ± 14 g / dl) than in women (12.1 ± 1.1 g / dl). However, there was no significant difference (p> 0.05) for serum ferritin levels, with the tendency of male serum ferritin levels (207.8 ± 159.0) were higher than women (141.5 ± 175.1) (attachment, Table 2).

Table 2: Serum Ferritin dan Hemoglobin level comparison based on Characteristics Demografi

Variabel		Serum Ferritin level		Hb level	
		Mean±SD	P	Mean±SD	P
Age (years)	<20 (n=9)	128,5±111,8	0,691*	13,2±1,2	0,866*
	21-30 (n=16)	212,3±213,3		13,7±1,9	
	31-40 (n=6)	197,5±174,4		13,7±1,8	
	>40 (n=6)	192,3±26,0		13,8±2,0	
sex	Men (n=25)	207,8±159,0	0,258*	14,3±1,4	0,001*
	Female (n=12)	141,5±175,1	*	12.1±1.1	*

**One-Way Anova; **Independent sample t test*

Comparative analysis of serum ferritin levels and hemoglobin based on dietary intake group using Mann Whitney test showed that there was no significant difference.Although, serum ferritin level in leprosy groupwith mean of 236.9 ± 204.9 were higher than control group with mean of 132.9 ± 85.4. Hemoglobin of leprosy groupwith mean of 13.1 ± 1.9 was lower than control group with mean of 14.2 ± 1.3, but this result was not statistically significant. There was significant difference (p <0.05) of Hb level based on food intake group; Hb level were higher in good intake (13.9 ± 1.6gr / dl) than in poor intake (11.5 ± 0.8 g / dl) (attachment, Table 3).

Table 3: Serum Ferritin leveland Hemoglobin levelcomparison based on group and food intake

Variabel		Kadar serum ferritin		Kadar Hb	
		Mean±SD	P	Mean±SD	p
Group	Leprosy(n=19)	236,9±204,9	0,245*	13,1±1,9	0,057*
	Control(n=18)	132,9±85,4		14,2±1,3	
Food intake	Good (n=33)	164,9±126,8	0,380*	13,9±1,6	0,004*
	Poor (n=4)	362,3±329,5		11,5±0,8	

**Mann Whitney;*

Based on combination of leprosy group and intake group results, this study showed that Hb levels were influenced by leprosy status and intake. Highest Hb concentration was observed in control subject with good intake (14.3 ± 1.3 gr%) and lowest on leprosy subject with poor intake (11.2 ± 07 gr%), with Mann Whitney test showed significant differences (p <0.05).

Spearman correlation test showed significant correlation (p <0.05) with correlation coefficient equal to r = 0.460, in combination of leprosy status and intake; indicates that the more the two factors are found (leprosy + poor intake), the lower Hb level (attachment, Table 4).

Table 4: Hemoglobin level comparisonbased onleprosy andfood intake combination

Kombinasi MH dan Asupan		Kadar Hb		P
		Min/maks (Med)	Mean (SD)	
Control+good food intake	n=17	12,0/15,9(14,6)	14,3(1,3)[aa]	0,025
Leprosy+ good food intake	n=16	9,5/16,3(13,8)	13,4(1,8)[ab]	
Control+ poor food intake	n=1	12,3	12,3[ab]	
Leprosy+poor food intake	n=3	10,5/11,8(11,2)	11,2(0,7)[bb]	

MH=….Kruskal Wallis+Mann-Whitney U test; SpearmanCorrelation

Comparison of serum ferritin levels based on combination of leprosy status and dietary intakeshowed that serum ferritin levels were not significantly affected (p> 0.05).

The serum ferritin levels of leprosy subjects with poor intake was high (476.8 ± 290.3) and low on non- leprosy subjects with good intake (139.6 ± 83.0), nonethelessit was not statistically significantsince serum ferritin in each group varies greatly. Moreover, Spearman correlation test showed a non-significant correlation (p>0.05) with correlation coefficient equal to r = 0,160; indicates that the more the two factors are found(leprosy + poor intake), the higher serum ferritin level (attachment, Table 5).

Table 5: Ferritin Serum level comparison based on leprosy and food intake

Kombinasi MH dan Asupan		Kadar Serum Feritin		P
		Min/maks (Med)	Mean (SD)	
Control+ good food intake	n=17	14,6/258,1(102,9)	139,6(83,0)	0,114
Leprosy+ good food intake	n=16	9,5/16,3(166,6)	191,9(159,6)	
Control+ poor food intake	n=1	18,7(18,7)	18,7	
Leprosy+ poor food intake	n=3	146,8/692,5(591,2)	476,8(290,3)	

Kruskal Wallis; SpearmanCorrelation (r=0,160 p=0,176)

4. Discussion

This study showed that mean hemoglobin level in leprosy patients was 13.1 ± 1.9 gr / dl, lower than control group 14.2 ± 1.3 gr / dl; serum ferritin levels were higher in leprosy patients with mean 236.9 ± 204.9 than control group 132.9 ± 85.4. Of 37 samples, the lowest hemoglobin level was 9.5 gr / dl and the highest was 16.3 gr / dl with mean of 13.6 ± 1.7 g / dl; the lowest serum ferritin level was 6.5 gr / dl and the highest 692.5 gr / dl with the mean of 186.3 ± 165.0 gr / dl.

The levels of hemoglobin basedon sex group showed hemoglobin level in men was higher with mean of 14.3 ± 1.4 gr / dl compared to women with mean of 12.1 ± 1.1 gr / dl, this result was proved significant with independent sample t test for hemoglobin level($p < 0.05$). Serum ferritin levels in males was higher with mean of 207.8 ± 159.0 ng / ml compared to women with mean of 141,5 ± 175,1 ng / ml, but statistically not significant. Based on the age group there was no significant difference in hemoglobin level, whereas the mean value of serum ferritin level at 21-30 years old was higher while the lowest was at under 20 years old.

There were more of male leprosy patients than female and the largest number of leprosy patients aged between 21-30 years old. The sex and age of all subjects,the leprosy and control group were homogeneous in this study, and no significant differences were obtained. This is in accordance with the literature which states that the prevalence of leprosy is higher in men than women, especially since men are more susceptible to lepromatous leprosy and tend to seek treatment [5]. Leprosy can affect all ages, the most encountered between 10-20 years and 30-60 years. Leprosy in adult population living in endemic area is often a reinfection or superinfection of an individual that previously infected but subclinicallyundergo decreasing immune response as they grow older.

Levels of hemoglobin in leprosy patients were lower with mean of 13.1 ± 1.9 gr / dl compared to control group with mean of 14.2 ± 1.3 gr / dl, while serum ferritin level of leprosy patients was higher with mean of236.9 ± 204. 9 ng / ml compared to control with mean of 132.9 ± 85.4, however these results were not significant statistically. Hemoglobin levels based on food intake in both groups of samples, hemoglobin levels were higher in good food intake with mean of 13.9 ± 1.6 g / dl compared to poor food intake with mean of 11.5 ± 0.8 gr / dl and there was a significant difference using the Mann Whitney test. A study reported that anemia is often found in patients with either tuberculoidor lepromatous leprosy, however anemia were not severe and no erythrocyte morphological abnormalities were found. Anemia is a condition of decreased function and quality of hemoglobin.

Control group with good food intake had higher hemoglobin level with mean of 14.6 gr / dl compared to leprosy patients with good food intake with mean of 13.8 gr / dl, and lowest hemoglobin level was found in leprosy patient with poor intake with an average of 11.2 gr / dl, this result was statistically significant using Spearman correlation test. Control group with good food intake had lower serum ferritin level with mean of 102.9 ng / ml compared to leprosy patients with good food intake with mean of 166.6 ng / ml, whereas in leprosy patients with poor food intake had the highest serum ferritin level at 591.2 ng / ml, but this result was not found significant. Nutrients play a role in the formation of red blood cells (erythropoesis)since iron (Fe), folic acid and vitamin B-12are needed for the process of erythopoesis. A person is at risk of anemia when there is shortage of these

substances. There are some interventions to prevent anemia, such as consuming foodthat rich in iron, food containing iron / heme is very bioavailable; especially red meat, poultry and fish. The body procures iron from food metabolism in the gut. Iron obtained from food is released in the stomach through the action of peptic enzymes and stomach acid. Iron passes through the duodenum, the site of maximum absorption of iron. Each iron feri (Fe3 +) will be converted to a form of fero (Fe2 +) by a membrane enzyme, cytochrome B in the duodenum. The ferrous iron can then bind to divalent metal in the membrane (divalent metal transporter-1) to enter the enterocytes. In order to be absorbed by the body, iron must pass through the enterocytes and then exit using feroportin then can be released to plasma transferrin. This process requires changing iron again into a feri shape with the help of some auxiliary proteins. If the removal of iron from enterocytes does not occur then iron is converted to ferritin. Ferritin will disappear along with the release of enterocytes into the intestinal lumen [6]. Leprosy is a chronic infectious disease caused by M. lepraethat primarily attacks peripheral nerves and may subsequently attack the skin, oral mucosa, upper airway, reticuloendothelial system, eyes, muscles, bones and testes except the central nervous system.Leprosy has a broad clinical spectrum that is related to the immunological response of the host, both natural immune responses and adaptive immune responses. The function of T cells and antibodies in the immunologic response to leprosy are influenced by the role of various cytokines [7]. Ferritin is the major protein associated with iron deposits by macrophages and hepatocyte cells. In chronic disease anemia there is a decrease in the number of reticulocytes and serum erythropoietin levels, iron concentrations may be normal to declined; serum transferrin levels may be decreased or normal; and serum ferritin levels may be normal or elevated. Anemia due to chronic disease occurs due to iron homeostais disorders which there isan increase in uptake and retention of iron in reticuloendothelial cells (RES). Iron will be stored in many RES cells hence the availability of iron for proliferation of erythroid progenitor cells, forerythropoetin production and the lifespan of red blood cells will be disrupted. According to WHO, cases of anemia occur in tropical and sub-tropical areas such as Asia and Africa, mainly cause by chronic conditions with long course of disease (iron deficiency or chronic disease) [8]. The proinflammatory stimulus also induces iron retention in the macrophages through resistance to feroportin expression thus no iron removal from macrophages occurs. IL-10 will accelerate the accumulation of iron in transferrin-enriched macrophages as well as stimulate the expression of ferritin. TNF-α will promote degradation and erythrocyte phagocytosis by macrophages. TNF-α, IL-1, IL-6, IL-10 together will stimulate the expression of ferritin, increasing iron storage and retention within the macrophages. There is also a blockage of erithropoetin production in the kidneys by TNF-α and IFN-γ. The availability of iron in the circulation will be limited with decreased activity of erythropoietin therefore inhibit erythropoesis resulting in anemia [9].

5. Conclusion

The authors concluded that hemoglobin levels in leprosy patients were lower than in the control group. Serum ferritin levels in leprosy patients were higher than in the control group. Leprosy patients with good food intake have higher hemoglobin levels than leprosy patients with poor food intake. While the control group with good food intake had higher hemoglobin levels than leprosy patients, with significant results. Researchers suggest that leprosy patients need to have good food intake. Further research is needed to assess hemoglobin and serum ferritin levels in leprosy patients after MDT-WHO therapy.

Acknowledgement

The authors would like to acknowledge to friends and family for supporting me during this study.

6. Competing Interest

The authors declare that they have no competing interests.

References

[1] Eichelmann K et al. (2013). Leprosy an Update: Definition, Pathogenesis, Classification, Diagnosis and Treatment. Actasdermosifiliogr. 104(7): 554-63.

[2] WHO.(2013). Weekly Epidemiological Record. 88(35): 365-380.

[3] Penna M.L.F et al. (2012).Primary Result of Clinical Trial for Uniform Multidrug Therapy for Leprosy Patients in Brazil (U-MDT/CT-BR): Reactions Frequency in Multibacillary Patients. Lepr Rev. 83: 308-319.

[4] Dogra S et al. (2013). Clinical Characteristics and Outcome in Multibacillary (MB) Leprosy Patients Treated with 12 Months WHO MDT-MBR: a Retrospective Analysis of 730 Patients from a Leprosy Clinic at Atertiary Care Hospital of Northern India. Lepr Rev. 84: 65-75.

[5] Correa R.G.C.F et al. (2012).Epidemiological, Clinical, and Operational Aspects of Leprosy Patients Assisted at a Referral Service in the State of Maranhao, Brazil. Rev Soc Bras Med Trop. 45(1): 89-94.

[6] Bain B.J. (2012). Hematologi Kurikulum Inti. Jakarta: EGC.

[7] Lee D.J et al. (2012). In: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K, editors. Fitzpatrick's dermatology in general medicine. 2. 8 ed. New York: McGraw-Hill.

[8] Herbinger K.H et al. (2013). Infection-Induced Anaemia: A Cross-Sectional Study of 14,636 German Travellers Aged 20-49 Years. Infection. (41).

[9] Sankaran V.G & Weiss M.J. (2015). Anemia: Progress in Molecular Mechanisms and Therapies. Nat Med. 21(3): 221-30.