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FULL PAPER

The differences in the results of haemoglobin level examinations in female adolescents using digital and automated hematology analyzer methods

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Abstract

The prevalence of anemia in Indonesian female adolescents is still high, which is around 32%. This means that 3-4 out of 10 female adolescents suffer from anemia. Anemia will greatly affect the next cycle of adolescents' health and productivity. Therefore, it is necessary to have routine screening to determine the prevalence of anemia. Routine screening is used to identify the incidence of anemia and can be done by examination of haemoglobin (Hb). The purpose of this study is to know the difference between Hb examination using the Digital (Hb meter) and automated hematology analyzer (AHA) with the sodium lauryl sulphate (SLS) method (AHA-SLS). The research method used was descriptive comparative with female adolescents as subjects from Maros, South Sulawesi. The results showed that in the digital method of Hb examination, 106 of 505 female adolescents were categorized as anemic. Meanwhile, in the AHA-SLS method, only 33 of 106 female adolescents suffered from anemia. Hence, there was a significant difference between the results of the Hb examination with the Digital method and the AHA-SLS method (p = 0.000)

1. Introduction

Iron deficiency anemia (IDA) is one of the most common hematological diseases found in infants, children and women of reproductive age, including adolescents. Children and female adolescents with IDA experience growth and development disorders, behavioral changes and motor disorders, which can reduce learning abilities and academics achievements in school. IDA is a type of anemia caused by a lack of iron availability in the body, causing insufficient iron needed for erythropoiesis. However, the need for iron is increased during growth, such as in infants, children, adolescents, pregnancy and breastfeeding (Kurniati, 2020)

IDA is the final stage of the progressive iron deficiency process, before iron depletion and iron deficiency. Active female adolescents have a high potential to suffer from iron deficiency. Iron deficiency in active female adolescents can be caused by high-intensity exercises or due to low iron intake in meals and loss of iron during menstruation. Iron deficiency and muscle mass also affect the bone density of adolescents (Pradita *et al.*, 2020).

Sunarsih *et al.* (2020) stated that the prevalence of anemia in female adolescents in the world is 4.8 million. Based on the results of the Basic Health Research (Riskesdas), the incidence of anemia in Indonesia is still high, at 32%, meaning 3-4 out of 10 female adolescents suffer from anemia. The frequency of iron deficiency in developing countries increases iron deficiency anemia by 2-5 times due to several factors such as infection and malnutrition, which can also be caused by nonoptimal nutritious intake and lack of physical activity.

Therefore, it is necessary to prioritize various policies to handle the cases of anemia in adolescents because it can break the cycle of reproductivity in pregnant women and impact the birth of babies, causing them to be born with low cognitive and leading to the incidence of stunting. According to Kurniati (2020), in the management of iron deficiency anemia, the selection of the right method for laboratory examinations related to anemia has an important role in screening, establishing a diagnosis, and monitoring the success of therapy.

The gold standard method for the identification of IDA is direct bone marrow biopsy stained with Prussian

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blue stain. However, this method is invasive when carried out routinely, so an indirect examination is usually carried out which includes hematological and biochemical examinations. Hematological examination is based on the appearance of erythrocytes, namely Hb and MCV, while the biochemical examination is based on iron metabolism, namely Zinc protoporphyrin (ZPP) and serum ferritin concentration (Kurniati, 2020).

Hemoglobin examination is more sensitive and can directly determine anemia than Hematocrit. The price of Hb examination is very affordable and the results are fast, so it is often used to screen for iron deficiency. However, the Hb examination is indeed not specific to IDA and is less predictive of IDA (Kurniati, 2020). According to the WHO, the definition of anemia is when the Hb level is less than 13 g/dL for men > 15 years of age, less than 12 g/dL for women > 15 years of age and less than 11 g/dL for pregnant women.

Digital hemoglobinometers, such as the Hb-Quick® and HemoCue® models have been widely used in anemia surveys in the population or for other clinical studies (Nkrumah *et al*., 2011; Gwetu *et al*., 2013; Hidayat and Sunarti, 2015; Qomarudin, 2016; Adam *et al.*, 2018, Mardiah *et al.*, 2019; Whitehead *et al.,* 2019; Nass *et al.*, 2020, Puspitasari *et al.*, 2020). This method is easy to use and able to obtain results quickly. The results of the research by Kusumawati *et al.*(2018) also showed that the examination using the Hb meter is more sensitive than the Sahli method, the results of which are very subjective.

According to Gwetu *et al.* (2013), the estimation of Hb examination using a Hb meter is quite good compared to the Gold Standard method, namely cyanmethemoglobin. This tool can be recommended for anemia screening and also iron deficiency anemia detection. The value of sensitivity range of 75-91%, specificity range of 88-100% and positive predictive values ranging from 75-80% for the detection of anemia. The mean difference in Hb from paired samples ranged from 0.2-0.35 g/dL (0.7%).

The newest and more advanced method to detect blood cell components and make a clinical diagnosis and identify blood disease is automated hematology analyzer (AHA) with the sodium lauryl sulphate (SLS) method. AHA is one of the advanced testing instruments that has been widely used in the field of medical testing, improving the efficiency and accuracy of testing (Chhabra *et al*., 2018; Wang *et al.*, 2019)

The SLS method is also widely used for screening and diagnosing anemia. The advantage of this method is the use of non-toxic and environmentally friendly oxidative reagents. In contrast to the gold standard method, cyanmethemoglobin, uses potassium cyanide as a reagent. This reagent is toxic at high concentrations so the waste is not safe for the environment.

SLS is a type of surfactant. The assumption is that SLS will convert hemoglobin into methemoglobin and then into oxyhemoglobin, hemochrome and methemoglobin through oxidative activity. However, this method does not require oxidative reagents and does not produce toxic waste such as KCN and NaN_3 which can cause environmental damage. This method is considered very applicable for manual procedures and Hemalog-8/90 as is usually done in clinical laboratories (Oshiro *et al.,* 1982).

This SLS method is proven to have a fairly high accuracy and similarity in estimation when compared to the cyanmethemoglobin method. The correlation value between the two methods is known to be quite strong, with r = 0.986 (Chakravarthy *et al.,* 2012).

This research is one of the early stages of a series of research on the efficacy of food products to prevent anemia for female adolescents. Subjects who took the food efficacy test were female adolescents aged 12-22 years who suffered from anemia. The purpose of this study was to determine the anemia status of prospective subjects for the efficacy test and to compare the results of the Hb level examination using the Hb meter and the AHA-SLS method.

2. Materials and methods

This study used a comparative descriptive research method with a cross-sectional approach. The purpose of this study was to determine the differences in the results of hemoglobin (Hb) examinations of female adolescents using the Hb meter method and the AHA-SLS method. The variables in this study were the examination of adolescents' Hb levels using a Hb meter and examination of Hb levels using the AHA-SLS method. The subjects of this study were female adolescents aged 12-21 years from Maros, South Sulawesi. The sampling technique used in this study was accidental sampling, with criteria for female adolescents who had experienced menstruation and were willing to become research subjects.

The tools and materials used for this research are capillary blood (fingertip) and approximately 5CC venous blood, EDTA, tube, syringe, tourniquet, cotton, alcohol, capillary pipette, Hb meter "*Quick Check*", micropipette, Automated Hematology Analyzer (SYSMEX XN-100).

The number of initial subjects in the study was 503

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female adolescents. The first Hb examination was carried out on 503 subjects using the Hb meter method. Subjects who were categorized as anemia from this examination were then checked for their Hb levels again using the SLS method. The data obtained were analyzed with SPSS for Windows, using the paired sample T-Test statistical test.

3. Results

The first Hb level examination was carried out on 503 female adolescent subjects according to inclusion factors. Examination of Hb levels is done by digital method or Hb meter. The results of the examination of Hb levels with an Hb meter showed that there were 106 subjects (21.07%) categorized as anemia with Hb levels < 12 g/dL. Meanwhile, the other 397 subjects (79.93%) were categorized as non-anemic, with Hb levels > 12 g/dL (Table 1).

Table 1. Hb examination results using Hb method

Anemia Status	Numbers (N)	Percentage (%)
Anemia	106	21.07
Non Anemia	397	78.93
Total	503	100

The next stage of this research was to examine Hb levels using the AHA-SLS method from 106 subjects who were categorized as Anemia from the previous Hb level examination. At the same time, the data collection related to the characteristics of subjects was carried out in more depth. Characteristics of subjects based on age can be seen in Table 2. which shows that the age of subjects varies greatly, with a minimum age of 12 years and a maximum age of 21 years. The age of the majority of subjects was 19 years old (23.58%), and at least 12 years old (2.83%).

Table 2. Characteristics of subjects by age.

Age(years)	Numbers (N)	Percentage (%)
12	3	2.83
13	4	3.77
14	5	4.72
15	5	4.72
16	12	11.32
17	11	10.38
18	22	20.75
19	25	23.58
20	15	14.15
21	4	3.77
Total	106	100

The results of the examination of Hb levels using the AHA-SLS method showed that there were fewer female adolescent subjects categorized as anemia, compared to the Hb level examination using the Hb meter method. Out of the 106 subjects with anemia tested using the Hb meter method, only 33 subjects (31%) were categorized as anemia from the AHA-SLS method. Meanwhile, 73 other subjects (69%) were categorized as non-anemic after having their Hb levels checked using the AHA-SLS method (Table 3).

Table 3. Hb examination results using AHA-SLS method.

Anemia Status	Numbers (N)	Percentage (%)
Anemia	33	31
Non Anemia	73	69
Total	106	100

Table 4 shows that there are differences in the average Hb levels of both Hb examination methods conducted on 106 female adolescents. The average Hb level of the subjects using the Hb meter method is 9.95 g/dL, while the average Hb level of the subjects using AHA-SLS method is 12.6 g/dL. The average Hb levels of the subjects from the Hb meter method appear lower than the Hb levels from the SLS method.

Table 4. Hb level examination results Hb meter and AHA-SLS methods

	Mean	N	Std. Deviation	Std. Error Mean
Hb meter	9.95	106	2.16	0.21
Hb- SLS method	12.6	106	1.53	0.15

The results of the statistical paired sample t-test show that there is a significant difference between the Hb levels of the Hb meter method and the Hb levels of the AHA-SLS method, with p-value = 0.000 ($p < 0.05$). These results can be seen in Table 5.

4. Discussion

In this study, the selection stage for anemia subjects in young women was carried out using the Hb meter and AHA-SLS methods. The Hb meter method was chosen because of fast testing, ease to use, efficiency and needle -free. Photometric Point of Care Testing (POCT), such as Hb-Quick® and HemoCue® models, provide Hb measurements within 10 seconds using approximately 10 µl of fresh arterial, venous or capillary blood

Table 5. Differences in Hb level examination with the Hb meter method and the AHA-SLS method.

		Paired Differences					t	df	Sig (2-tailed)
		Mean	Std.Dev	Std.Err Mean	95% Conf Intv Diff Lower	Upper			
Pair 1	Hbmeter-SLS	-2.66	2.85	0.28	-3.21	-2.11	-9.6	105	0

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(Qomarudin, 2016, Whitehead *et al.*, 2019). In addition, a digital Hb meter or POCT system is also recommended by the World Health Organization (WHO) in conducting a survey of the prevalence of anemia in the population (Nkrumah *et al.*, 2011). The results showed that the Hb level examination using the Hb meter method gave a low average of Hb level, so many subjects were categorized as Anemia. However, after examining Hb levels using the AHA-SLS method, the number of subjects categorized as anemia decreased to 33 subjects (6.56%) from 106 subjects (21.07%). The average Hb level from the AHA-SLS method (12.6 g/dL) was higher than the Hb meter method (9.95 g/dL). This shows that the sensitivity of the AHA-SLS method to measure Hb levels is higher than the Hb meter. In daily laboratory practice, the use of AHA-SLS method with modification (SYSMEX XN-10 or XN-100) provides reliable results for diagnosing iron deficiency anemia in patients (Nivaggioni *et al.*, 2020; Velizarova *et al*., 2021)

The results of the research done by Nass *et al*. (2020) showed something different. The measurement of Hb from Hb meter (Hemocue® and Aptus®) in Gambian children gave a higher value than the AHA-Medonic® (overestimated value). This is thought to be due to the different types of blood drawn. The Hb meter uses capillary blood while the AHA uses venous blood. Meanwhile, another research showed that there was no agreement between HemoCue® and AHA, using either capillary or venous blood samples (Adam *et al.*, 2018)

Chaudhary *et al*. (2017) reported that there are several things that can affect the results of Hb examination, such as the source of the sample (finger or earlobe). When blood is sampled from the earlobe it produces data that is overestimated and has a very wide variation compared to data from the fingers. Next is the venous and capillary blood, postural effects and biological differences. In addition, the factors of analyst error during blood taking and when conducting examinations also affect the test results.

The mechanism of the digital method (Hb meter) is by calculating Hb levels in samples based on changes in electrical potential formed briefly which are influenced by chemical interactions between samples measured with electrodes on reagent strips (Akhzami *et al.*, 2016). Prasetya *et al.* (2016) reported that the procedure for taking blood has an effect on the final result of a blood test. Capillary blood sampling is sometimes carried out by massaging first, causing the cell fluid to come out and mixed with blood so that the blood is more watery than venous blood. Thus, capillary blood samples that are dilute or the amount is not in accordance with the standard, causes the value of the Hb level measured in the device to be lower than the actual value.

According to Hidayat and Sunarti (2015), the sensitivity of the Hb meter compared to the cyanmethemoglobin method is low, which is around 45.45%. This means only 45.45% of anemia sufferers are easily detected by using the Hb meter. Meanwhile, the Ministry of Health reported that a diagnostic tool is said to have good sensitivity if the value is 85%. In addition, the specificity value of Hb meter is 64.61%. This shows that the Hb meter method has poor specificity. According to Hidayat and Sunarti (2015), it is reported that ideally, a test tool that functions very well should have a fairly high specificity, which is close to a value of 100%.

Chakravarthy *et al.* (2012) reported that the SLS method was proven to have similarity and good accuracy compared to the Gold Standard method, cyanmethemoglobin. The correlation value between the two methods is known to be quite strong, with r = 0.986. In addition, the SLS method can prevent environmental pollution due to toxic waste produced from cyanide reagents from the cyanmethemoglobin method.

4. Conclusion

There is a significant difference between the results of the examination of Hb levels using the digital method (Hb meter) and the AHA-SLS method. The average Hb level from the Hb meter method is lower than the average Hb level from the AHA-SLS method. But, Hb meter is still can still available for Anemia screening. Further research is recommended to compare Hb levels with vena blood in various methods.

Conflict of interest

The authors declare no conflict of interest.

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