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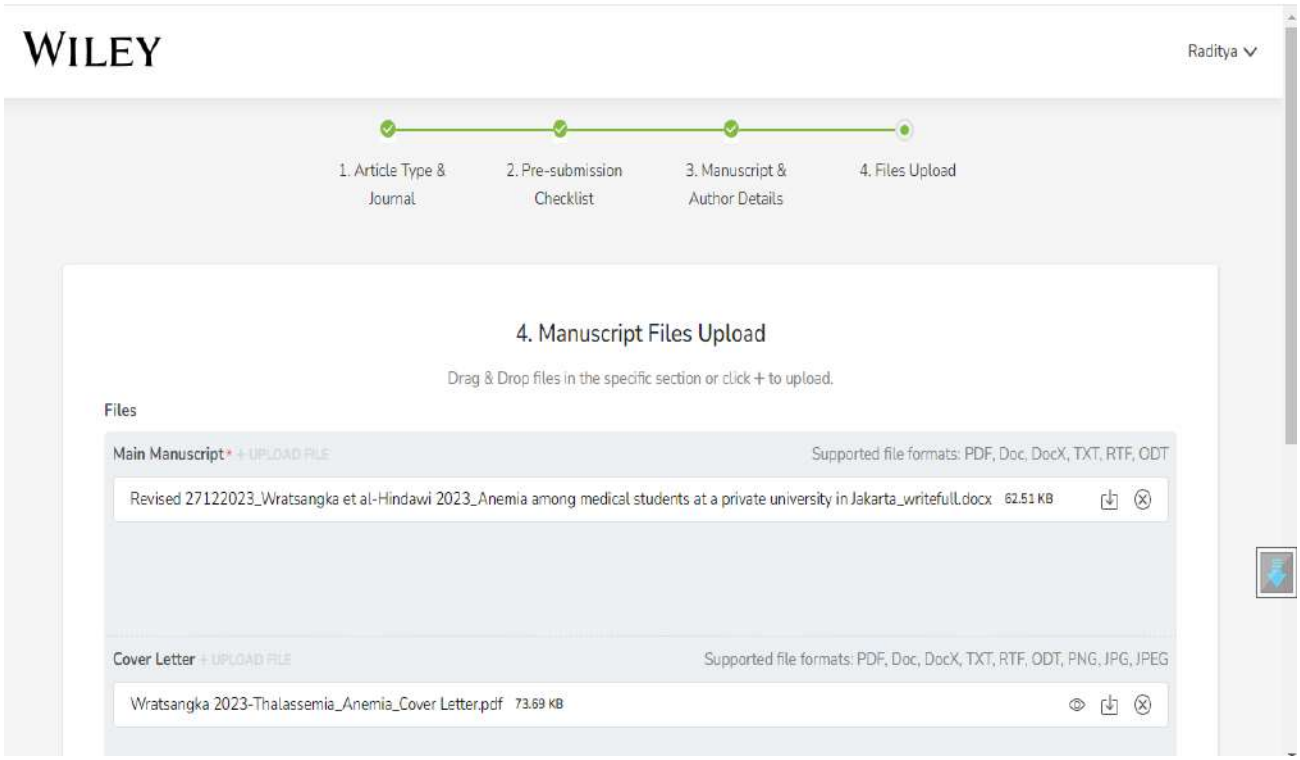

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Anemia among medical students at a private university in Jakarta:

Iron deficiency or carrier thalassemia?

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ABSTRACT

BACKGROUND: Anemia, a global health concern, affects one-fourth of the global population, particularly women. In Indonesia, its prevalent is at 23.7%, with 32.0% among 15–24 year-olds. Factors include poor nutrition, infectious diseases, chronic diseases, inherited disorders, and inadequate healthcare access. This study aimed to investigate anemia prevalence and its etiology among medical students in a private university.
METHODS: This study was a prospective descriptive research with a cross-sectional approach. Undergraduate students aged 18-23 years old were selected and consented to participate by a consecutive non-random sampling methods. Laboratory blood data were evaluated (including Hb, MCV, MCH, HbA_2, and Ferritin levels) and DNA was isolated to confirm the type of thalassemia carrier.
RESULTS: In total, 140 medical students, mainly female, were recruited. Anemia was found in 13.6% (11.4% had low MCV and/or MCH), and 16.5% had low MCV and/or MCH without anemia. Hb electrophoresis revealed high HbA_2 values, suggesting the HbE variant (2.1%), and β-thalassemia carrier (0.7%). DNA analysis confirmed *the cd26 mutation* and *heterozygous IVS1nt5*. Among those without anemia, 5% had *α-deletion*, while in the group with anemia, 1.4% had *α-deletion* (with coexistent IDA), 3.6% had *α-deletion*, and 0.7% had *β-mutation*.
CONCLUSION: DNA analysis can identify specific mutations associated with alpha-thalassemia, distinguishing between iron deficiency anemia and the alpha-thalassemia trait. Thalassemia screening should involve low MCV and/or MCH values as the first step (stage 1), followed by Hb analysis (stage 2) and DNA analysis (stage 3). In common areas, a combination of Hb and DNA testing is best. However, healthcare professionals must diagnose and treat thalassemia, as proper management relies on accurately identifying the underlying condition.

INTRODUCTION

Anemia is a worldwide public health issue, affecting both developed and developing nations. Anemia is also the third leading cause of years with lived disability (YLDs).[1] One-fourth of the global population is estimated to be anemic, with cases increasing rapidly in women. Over half a billion women between the ages of 15 and 49 make up the global anemia prevalence in 2019, including women of reproductive age (WRA; 29.9%), pregnant women (36.5%) and non-pregnant WRA (29.6%), with sub-Saharan Africa and South Asia having the most significant rates.[1] In 2021, there is an increase of 420 million cases over three decades.[1,2] Data in Indonesia revealed that the prevalence of anemia in Indonesia was 23.7%.[3] Additionally, the prevalence of anemia among individuals aged 15-24 years was 32.0%.

The high prevalence of anemia among young adults in Indonesia may differ among regions and population subgroups and is likely due to a combination of factors, including poor nutrition, infectious diseases, chronic diseases, inherited disorders (sickle cell anemia, thalassemia), and inadequate access to healthcare.[4,5] Interestingly, recent evidence has shown that the awareness programs and laboratory examinations have identified more thalassemia carriers, minors, or traits among young adults..[6]

Thalassemia carrier has a small red blood size (microcytic) as shown in low MCV values and/or less red blood cell color (hypochromic) as shown in low MCH values. Thalassemia carrier does not necessarily have low Hb. Anemia and thalassemia can cause low levels of hemoglobin in the blood, leading to low levels of red blood cells and decreased oxygen delivery to tissues.[7] Thalassemia is one condition that can cause anemia, however, thalassemia minor can be found without anemia.[7] The two conditions share some similarities, but have some essential differences.

Thalassemia is an inherited autosomal recessive gene disorder caused by a decreased or absent production of one or two types of globin chains, and classified according to the particular globin chain affected, such as α-globin and β-globin. Thalassemia is also a significant health issue in Indonesia, particularly among young adults.[8,9] Indonesia is located along the 'Thalassemia Belt', a geographic region that stretches from the Mediterranean to Southeast Asia, where the prevalence of thalassemia is highest.

Medical students, as part of the young adult population, are obliged to study various diseases, including anemia and thalassemia in their courses on genetics, hematology, and other related topics. Medical students' knowledge, attitudes, and practice towards thalassemia are likely to be shaped by various factors, including their academic and personal backgrounds, their exposure to patients with thalassemia, and their understanding of the social and cultural contexts in which the disease occurs. Medical students who look physically fit may not even know their status of carriers of thalassemia traits. Study in Malaysia involving 400 undergraduate medical students has reported that 14.5% students had hypochromic microcytic red cell indices, 11% showed thalassemia red cell indices, and only 3.5% had iron deficiency (IDA) red cell index which were finally confirmed by serum iron/TIBC analysis.[10]

Therefore, it is interesting to also explore the etiology of anemia among students in Indonesia compared to the neighboring country Malaysia. The study aimed to explore the anemia prevalence and the etiology among medical students from a private university in Jakarta.

MATERIAL AND METHOD

Study design

This study was a prospective descriptive research with a cross-sectional approach to explore the anemia prevalence among medical students and the etiology of the anemia. Laboratory

blood data were evaluated (including Hb, MCV, MCH, HbA_2, and Ferritin levels) and DNA was isolated to confirm the type of thalassemia carrier (trait). The study was approved by The Research Ethics Committee of the Faculty of Medicine, Universitas Trisakti (No. 011/KER/FK/IV/2021).

Subjects

Undergraduate students of the Faculty of Medicine, Universitas Trisakti, aged 18-23 were selected and consented to participate by a consecutive non-random sampling methods. Those who had a history of chronic disease and had received blood transfusions in the past three months were excluded from this study.

Complete blood count and HbA_2 measurement

Venous blood sampling was collected in a 3 ml EDTA tube to be checked further for a complete blood count (CBC), followed by Ferritin measurement and HbA_2 determination by High Performance Liquid Chromatography (Bio-Rad). CBC was analyzed using the Flow Cytometry Method Using a Semiconductor Laser (Sysmex-XN®); ferritin measurement was analyzed using the chemiluminescent magnetic microparticle immunoassay (CMIA) method (*Architect® i2000*). The definition of anemia is according to WHO standards based on sex (men <13g/dL and women <12g/dL). The red blood cell (RBC) indices were then analyzed based on a guide from the Ministry of Health, Republic of Indonesia, MCV< 80 fL and MCH < 27pg values which were used as initial thalassemia screening. Furthermore, Hb analysis data were classified based on HbA_2 fraction values according to the protocol of the manufacturer (Bio-Rad) where < 2.0% suggested for α-thalassemia carriers; 2.0–3.5% as normal value; >3.5% was indicated as β-thalassemia carriers; and very high HbA_2 level peak shown indicated HbE carriers. Hereafter, subjects who had normal HbA_2 but had low/normal Hb and/or low MCV and/or low MCH were examined further for DNA analysis. Those with low MCV and/or MCH and with

normal HbA2 value (2-3.5%) were further confirmed with DNA examination to distinguish the etiology of anemia whether due to iron deficiency or alpha thalassemia carrier. The students were also checked for their ferritin levels to determine whether anemia was due to iron deficiency

DNA genotyping identification

Subjects with low MCV and/or MCH, low and normal Hb, and low and normal ferritin were identified by the level of HbA2 and subsequently confirmed their genotype by DNA analysis (N = 39). Due to the limited availability of examination kits, DNA tests were carried out on 30 of 39 subjects, whose selection was done by simple random sampling.

We used the α-Globin StripAssay® (Ref. 4-160, ViennaLab Diagnostics GmbH) to detect 21 common α-globin mutations in separated teststrips. The procedure includes three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers, (3) hybridization of amplification products to teststrips containing allele-specific oligonucleotide probes immobilized as an array of parallel line. The bound biotinylated sequences on specific wild types and mutants are detected using streptavidin-alkaline phosphatase and color substrates. We prepared three separate PCR mixtures (A1, A2, and B), following both PCR conditions and hybridization according to the manufacturer's instructions. We analyzed the hybridization results by comparing the line with the provided CollectorTM sheet. In heterozygous conditions, both mutated bands and normal bands will appear. In homozygous mutations, only the positive band is colored. If no mutations are contained in the StripAssay panel, only PCR and normal controls are colored. Furthermore, to identify β-globin gene mutations, we used the β-Globin Strip Assay® SEA (Ref. 4-150, ViennaLab Diagnostics GmbH). The assay covers 22 common β-globin mutations in one strip only. Unlike the assay for α-thalassemia, the assay for β-thalassemia was only in one PCR mixture and followed the manufacturer's instructions.

The hybridization procedure and analysis of teststrips results were the same as in α-globin gene mutation detection.

RESULT

In total, 140 medical students were recruited, primarily female (69.3%; n=97), with a median age of 20 (range 18–26 years). Anemia was found in 13.6% (n=19) of the female students (table 1). Interestingly, 16.5% of students (n=23; 18 females and 5 males) were not anemic but had low MCV and/or low MCH. These students were subjected to the next level of the thalassemia screening program as indicated by the Ministry of Health. In addition, 16 (11.4%) students who were anemic and had low MCV and/or MCH were examined further to determine the etiology of anemia.

Table (1). Hemoglobin level based on gender and MCV and/or MCH level in medical students (n=140)

	Level of Hemoglobin		
	Not Anemia n (%)	Anemia n (%)	Total n (%)
Male	43 (30.7)	0	43 (30.7)
MCV and/or MCH			
• Normal	38 (27.1)	0	38 (27.1)
• Low	5 (3.6)*	0	5 (3.6)
Female	78 (55.7)	19 (13.6)	97 (69.3)
MCV and/or MCH			
• Normal	60 (42.9)	3 (2.1)	63 (45.0)
• Low	18 (12.9)*	16 (11.4)**	34 (24.3)

Note: Anemia Hb: <13.0 g/dL (male); < 12.0 g/dL (female)
Low MCV: <80 fL
Low MCH: <27 pg
* Not anemia but with low MCV and/or MCH, subjected for further examination
** Anemia and were further examined to explore the etiology of anemia

In our study, the students were examined by Hb electrophoresis (level 2), resulting in a very high HbA2 value (>13%), suggesting an HbE variant (2,1%; n=3); and a high HbA2 value

(>4% to 13%), indicating β-thalassemia carrier (0.7%; n=1); that was confirmed by DNA analysis (level 3), resulting in cd26 mutation and heterozygous IVS1nt5, respectively (table 2).

Table (2). Complete blood count examination (Level 1) followed by Hb variant A2 (Level 2) and DNA confirmation (Level 3) among medical students from a private university in Jakarta (n=140)

Level 1		Level 2		
MCV and/or MCH	Hb level	HbA2		
		2 – 3.5%	> 3.5%	>>> 13%
		n (%)	n (%)	n (%)
Low (n=39)	Not Anemia	20 (14.3) ***	0	3 (2.1) *
	Anemia	15 (10.7) ****	1 (0.7) **	0
Normal (n=101)	Not Anemia	98 (70.0)	0	0
	Anemia	3 (2.1)	0	0

Note:
*Cd26 (G>A) heterozygote;
**IVS1nt5 heterozygote;
***Further DNA analysis needed whether iron deficiency anemia or alpha Thalassemia carrier
****Need iron therapy first

Among those without anemia (n=15), 5% (n=7) had α-deletion (table 3). Furthermore, in a group with anemia (n=14), α-deletion (with coexistent IDA) was detected in 1.4% (n=2), αdeletion 3.6% (n=5), and β-mutation in 0.7% (n=1).

Table (3). Evaluation of Ferritin among students (n=30) with low MCV and/or MCH (Level 1) in relation to Hemoglobin level, HbA2 (Level 2) and DNA genotype (Level 3)

Level 1 (Low MCV/MCH) N = 30	Level 2 Hb Electrophoresis ($Hb A_2$) 2-3.5	> 3.5	>>>	Level 3 DNA Genotype
• **Not Anemia** (n = 16)				
• Ferritin Low (n = 1)	1			Beta (β): IVS1-nt5 [G>C] heterozygote + coexistent IDA ***)
			3	Confirmed HbE: codon 26 [G>A] heterozygote
• Ferritin Normal (n = 15) *)	4			No alpha (α) deletion or mutation found, need further exploration
	4			Alpha (α): Single α globin gene deletion 3.7 kb type heterozygote
	3			Alpha (α): Double α globin gene deletion SEA type heterozygote
• **Anemia** (n = 14)				
	4			No-α deletion or mutation; suggesting IDA
• Ferritin Low (n = 6)	1			Alpha (α): 3.7 kb triplicated α gene heterozygote + coexistent IDA ***)
	1			Alpha (α): α2 cd 59 [GGC>GAC] heterozygote + coexistent IDA ***)
	3			Alpha (α): Double α globin gene deletion SEA type heterozygote
• Ferritin Normal (n = 8) **)	1			Alpha (α): α2 cd 142 [TAA>CAA] (Hb Constant Spring) heterozygote
	1			Alpha (α): α2 cd 59 [GGC>GAC] heterozygote
		1		Beta (β): IVS1-nt5 [G>C] heterozygote

Note. Anemia was designated as Hb < 12.0 g/dL for females and Hb < 13.0 g/dL for males
*) One sample DNA examination failed
**) Two samples DNA examination failed
***) Point of interest; IDA with coexistence confirmed Alpha (ά)

DISCUSSION

Our study has shown that among medical students aged 18 to 23 years old, anemia has been detected in 13.6% (n=19) students, all females. Interestingly, 16.5% of students (18 females and 5 males) who are not anemic, have low MCV and/or MCH. All these students have been examined for ferritin, Hb electrophoresis, and DNA genotyping for alpha and beta-globin to explore the etiology of anemia.

Iron Deficiency Anemia or Thalassemia Carrier?

Indonesia, located in the thalassemia belt area, has many thalassemia careers in Southeast Asia, making anemia in young adulthood a significant concern. Anemia in young adults can have other causes, such as hemoglobin abnormalities or hemoglobinopathy, as the

most common causes. Efforts to detect early thalassemia careers have not been carried out systematically. Around 3.0-10.0% of the Indonesian population carry β-thalassemia (β-thal) and 2.6-11.0% of the population carry α-thalassemia (α-thal), with the highest incidence rates reported in regions such as Bali, East Nusa Tenggara, and Maluku[11] It is estimated that around 2500 babies are born with β-thal major (β-TM) each year.[11]

Medical students who have a middle-class socio-economic status and are knowledgeable about anemia and thalassemia should be aware of the effects of poor nutrition and inadequate dietary iron intake. Not consuming enough iron-rich foods (including red meat, poultry, fish, beans, lentils, tofu, fortified cereals, and green leafy vegetables) in their diet can lead to IDA, which is assumed to be low. However, the results showed that 13.6% (n=19) had anemia and additionally, 16.5% (n=23) were not anemic but had low MCV/MCH. Further analysis with Hb electrophoresis has shown that it is difficult to distinguish between IDA and alpha-thalassemia carriers. Both conditions can lead to microcytic (small red blood cells) and hypochromic (pale) red blood cells, making it necessary to perform additional tests, such as DNA examination, to make a definitive diagnosis. In restricted facilities, MCV<80 fl and/or MCH<27 pg is the optimal first-round mass screening approach for β-thalassemia carriers. Gradually implement Hb electrophoresis, and DNA analysis should be done in many regions to confirm mutations for accurate carrier detection wherever possible.[12]

The prevalence of anemia among medical students varies depending on numerous factors, but being knowledgeable in medical science and having a sufficient socio-economic background might be caused by other than IDA. A study of medical students from other parts of Indonesia has shown that only 7.9% are anemic, similar to other studies in Malaysia. The low prevalence of anemia is also found in Malaysia among Malay (6.6%), Chinese (6.9%), and Indian (26%).[10] Moreover, among medical students, anemia was observed in 33.4% of Pakistani students[13], in 28.3% of Indian students from Bangalore[14], and 52.7% in Nepal.[15]

Some studies mainly use CBCs from venous blood, while others use finger-pricking methods. These methods can produce different outcomes due to differences in hemoglobin measurement.[16] Venous blood is preferred for precise and accurate measurements of hemoglobin levels and other blood parameters. Fingerstick tests are used for point-of-care testing, rapid screenings, and immediate findings. However, they may need to be more precise in detecting blood disorders or making life-saving medical decisions. Medical experts often use venous blood testing for verification and more precise evaluation when clinical concerns arise.

Medical students, like many other young adults, can be susceptible to anemia due to dietary habits, lifestyle choices, and stress. The high cost of medical education at least indicates that their socio-economic status is quite good, however, they often have demanding schedules and face high levels of stress, which can contribute to poor eating habits, irregular meals, and inadequate nutrition. Moreover, prolonged studying periods and lack of physical activity can impact their health. Promoting healthy lifestyles, providing nutritious food options, encouraging physical activity, and raising awareness about maintaining good health can help mitigate the risk of anemia among medical students.

Anemia affects more women than men, especially those of reproductive age, and is more prevalent in areas with high iron deficiency or parasite diseases. Female adolescents have lower hemoglobin levels than male adolescents, with anemia prevalence in women being 33.7% compared to 11.3% in men during reproductive years.[1] It was largely due to factors like menstrual blood loss in females and hormonal differences between the genders. Menstrual blood loss indeed contributes to lower hemoglobin levels in females compared to males. Testosterone, higher in males, doesn't directly impact hemoglobin levels but may reflect differences in overall health. However, it's essential to note that lower hemoglobin levels in

females aren't always indicative of underlying health issues; they can be a natural consequence of menstruation.

Male adolescents have higher testosterone levels, which typically rise during puberty, contributing to physical changes like muscle growth, deepening of the voice, and the development of male sexual characteristics. While testosterone itself doesn't directly cause anemia, certain conditions associated with hormonal imbalances or other health issues might impact hemoglobin levels in males. Anemia in male adolescents can occur due to various reasons unrelated to testosterone levels. Iron deficiency, chronic diseases, certain genetic conditions affecting red blood cell production, or nutritional deficiencies can lead to anemia in males.

Thalassemia is a genetic blood disorder that affects hemoglobin synthesis. Thalassemia trait refers to thalassemia carriers with one defective and one normal hemoglobin gene. This defect results in reduced production of normal hemoglobin, leading to smaller and paler red blood cells (low MCV/MCH) but with normal overall hemoglobin levels (normal Hb). Microcytic anemia can occur if the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are both low. Iron deficiency, which can result from insufficient food intake, malabsorption, chronic or persistent blood loss, or increased demand for iron during pregnancy, is the most prevalent type of microcytic anemia. Other causes of microcytic anemia include hemoglobinopathy and sideroblastic anemia, a rare condition where the bone marrow produces abnormal red blood cells.

Difficulty to distinguish IDA and alpha-thalassemia trait

Iron deficiency anemia (IDA) and alpha-thalassemia trait (TT) are common conditions of microcytic hypochromic anemia (MHA). Distinguishing IDA and the alpha-thalassemia trait can be challenging due to their similar symptoms and laboratory findings. Iron therapy, usually

oral or intravenous iron supplementation, is the primary treatment for IDA. Monitoring the patient's response to iron therapy is crucial.[17] Alpha-thalassemia is a genetic condition affecting alpha-globin chain production in hemoglobin. Alpha-thalassemia trait carriers typically have mild or no symptoms, and their hemoglobin levels are usually within the normal range. The alpha-thalassemia trait does not require iron therapy, as it is unrelated to an iron deficiency. To differentiate between IDA and the alpha-thalassemia trait, healthcare providers often use various diagnostic tools, including blood tests, hemoglobin electrophoresis, and DNA analysis.[18] Hemoglobin electrophoresis can help identify the specific type of hemoglobin and reveal the presence of alpha-thalassemia. DNA analysis, such as polymerase chain reaction (PCR) or genetic testing, can definitively diagnose of alpha-thalassemia by detecting specific genetic mutations associated with this condition. If there is uncertainty in the diagnosis or a need for confirmation, further DNA examination can be valuable in determining the presence of alpha-thalassemia or other hemoglobinopathies. Both IDA and the alpha-thalassemia trait can coexist, making a precise diagnosis even more important.

Limitations of the study

The study's limitations include limited availability of examination kits for subjects with anemia and low MCV/MCH, and no iron therapy was given to those suspected of IDA. Iron therapy can be used as a diagnostic tool to help distinguish between IDA and the alpha-thalassemia trait, especially when there is uncertainty in the diagnosis. Close monitoring of the patient's response to iron therapy is crucial during the diagnostic trial. Self-administering iron supplements without proper diagnosis and supervision is not advisable, as excessive iron intake can have adverse health effects.[19]

CONCLUSION

Thalassemia is a genetic blood disorder characterized by abnormal hemoglobin production that results in reduced production of hemoglobin and red blood cells, leading to anemia. Low MCV and/or MCH (stage 1) are common indicators observed in thalassemia, but hemoglobin electrophoresis (stage 2) and DNA genotyping (stage 3) are two essential tests needed in diagnosing thalassemia. Genetic testing can identify specific mutations or changes in the genes responsible for hemoglobin production to confirm the presence of thalassemia and determine its type (alpha thalassemia or beta thalassemia) and severity. DNA analysis can also distinguish between iron deficiency anemia and the alpha-thalassemia trait, which has many similarities. In places where thalassemia is common, combining Hb analysis and DNA testing as the second and third stage tests is the best technique to determine a person's career status. However, a healthcare professional must make the diagnosis and treatment plan, as proper management depends on accurately identifying the underlying condition.

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Authors' contributions:

RW contributed in the study concept, secured grants for study funding, designing the study, recruitment of respondents, data analysis, and drafting the manuscript. IMN designing and conducting lab work for genetic analysis. EXT and AK managed the data collection and performed the statistical analysis. All authors took part in compiling references and contributed

to constructing the research plan and giving insight into research implications for the near future study and policy, and finalizing the draft manuscript.

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The author declares that there are no significant competing financial, professional, or personal interests that might have affected the performance or presentation of the work described in this article.

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Dr. dr. Raditya Wratsangka, Sp.OG-K <raditya@trisakti.ac.id>

4215439: Manuscript returned to draft

Anemia <anemia@hindawi.com> Wed, Dec 27, 2023 at 2:58 PM
Reply-To: Kisslena Ferreras <kferreras@wiley.com>
To: Raditya Wratsangka <raditya@trisakti.ac.id>
Cc: John-Lenard Matel <lmatel@wiley.com>

WILEY

Dear Dr. Raditya Wratsangka,
Your manuscript "Anemia among medical students at a private university in Jakarta: Iron deficiency or carrier thalassemia?" with ID 4215439 submitted to Anemia has been returned to draft. We found the following issues which need to be resolved:

- **undefined**
  - 1.) There is a difference between the manuscript's affiliation on the system and the PDF file. Please confirm the final affiliation and Kindly update your records and ensure that all data provided in the system should be matched in your main PDF file.
  - Upon checking your manuscript we noticed that an existing source is not cited in the manuscript. You can check the aforementioned source at: "https://inabj.org/index.php/ibj/article/view/1960" Please can you ensure to cite any original sources and add to your references? Kindly update your manuscript file in the system.

> *This paper has been returned to draft for the reasons listed above. Please could you log into your account in https://review.hindawi.com/login and address these points as soon as possible? The changes should be made on this submission 4215439, please do not submit a new manuscript.*

In order to be considered further, follow the link below, and complete and submit with the required updates.

MANUSCRIPT DETAILS

Kind regards,
Kisslena Ferreras
Anemia

Cover Letter

Raditya Wratsangka
Department of Obstetrics and Gynecology
Faculty of Medicine, Universitas Trisakti
Kampus B – USAKTI
Jalan Kyai Tapa 260, Grogol,
Jakarta 11440 - Indonesia

December 27, 2023

Dear Editorial Board of *Anemia*

We would like to submit a new manuscript entitled "**Anemia among medical students at a private university in Jakarta: iron deficiency or carrier thalassemia?**" for consideration by the *Anemia*.
We confirm that this work is original and has neither been published elsewhere nor is currently under consideration for publication elsewhere.

Anemia is a worldwide public health issue, affecting both developed and developing nations, including Indonesia. Data from Indonesia revealed that the prevalence of anemia in Indonesia was 23.7%. Additionally, the prevalence of anemia among individuals aged 15–24 years was 32.0%. Thalassemia, as one of the causes of anemia, is also a significant health issue in Indonesia, particularly among young adults. Indonesia is located along the 'Thalassemia Belt', where the prevalence of thalassemia is highest. Medical students who look physically fit may not even know their status as carriers of thalassemia traits. The prevalence of anemia among medical students (as well as young adults) varies depending on numerous factors, but being knowledgeable in medical science and having a sufficient socio-economic background might be causes other than IDA.

Efforts to detect early thalassemia careers in Indonesia have not been carried out systematically. Interestingly, recent evidence has shown that awareness programs and laboratory examinations have identified more thalassemia carriers among young adults, including the detection of thalassemia carriers in non-anemia subjects. Our paper presents the hematology's profile of medical students at a private university in Jakarta, Indonesia, the prevalence of anemia and the etiology of anemia, and furtherly distinguishes IDA from alpha-thalassemia carriers.

We hope the results and discussions of the research in this paper are consistent with the scope of *Anemia* and can raise public awareness and efforts to examine the carriers of thalassemia more systematically.
Please address all correspondence concerning this manuscript to me at raditya@trisakti.ac.id

Thank you for your consideration of this manuscript.

Sincerely,

[Raditya Wratsangka]

UNIVERSITAS TRISAKTI

Dr. dr. Raditya Wratsangka, Sp.OG-K <raditya@trisakti.ac.id>

4215439: Revision requested

Anemia <anemia@hindawi.com> Mon, Jan 29, 2024 at 3:19 PM
Reply-To: Anemia <lmatel@wiley.com>
To: Raditya Wratsangka <raditya@trisakti.ac.id>

WILEY

Dear Dr. Raditya Wratsangka,

In order for your submission "Anemia among medical students at a private university in Jakarta: Iron deficiency or carrier thalassemia?" to "Anemia" to proceed further in the review process, you will need to revise your manuscript.

Reason & Details:

> “
>
> *Dear Author,*
> *Could you improve your article according to two reviewer's suggestion please?*
> *Best regards*

When you have finished revising, follow the link below to submit your revision:

MANUSCRIPT DETAILS

Kind regards,
John-Lenard Matel
Anemia

Reviewer Comments:

> “
>
> ***Reviewer 1 Comments to the Author***
>
> > *Dear thours have some suggestions and recommendations to improve the quality of the current manuscript as follows:1- In the study title (Anemiaamong medical students at a private university in Jakarta:Irondeficiency or carrier thalassemia?). It's better to remove the Private university because if the university is private of government it does not matter and it has no any significant impact because already your target are students and those students are the same in both universities if it is private or governmental.2- In study design you mention that (This study was a prospective descriptiveresearch). prospective study usually you examine something (diseases) before it will happen, like monitoring and following the patient till the disease or outcome appears. But here you come and take the sample the anemia already occurs and also mutation already was inherited it seems to be retrospective. Before you make any decision please go and read what is the difference between prospective and retrospective study.Most of people are confusing here and they think that if you meet patient and take sample from them this is prospective but the fact is if you conducted the study before the disease occur this is prospective and if you conducted the disease after this is*

retrospective. 3- How you select the 140 medical students as your sample size. which the formula used to calculate the sample and this number is less for this study type.4- In your result and limitations i saw the majority of cases are female and we know all parameters related to RBC are less in female when we compare with male . Do these differences not affect your finding? and you need to mention this in your limitations.5- The reference numbers (19) are less for this study. Please cite more references.

Reviewer 2 Comments to the Author

This manuscript is interesting. However, there are some issues needed to be clarified.
- Methods - please mention more about the cutoff for serum ferritin to diagnose iron deficiency anemia in this study.
- Methods - Since you use BioRad Variant to diagnose thalassemia, please mention why this study use cutoff of Hb A2 >3.5% to diagnose beta-thalassemia trait instead of 4% as suggested with machine
- Methods - the regular cutoff for diagnose Hb E by BioRad Variant is Hb A 25-35%, if Hb A is between 10-25%, it usually means that this patient have both alpha-thalassemia (trait/disease) with Hb E trait. Please discuss more particularly with the cutoff of Hb A2 at >13%
- Results - patients with anemia but have normal MCV/MCH, what could be the cause? Please discuss more.
- Results - patients who have low MCV/MCH but normal ferritin and DNA for alpha- and beta-globin genes, what could be the cause? Please discuss more.
- Results - could the authors show the level of Hb in this study? as it could affect the symptoms/signs or quality of life in this population.
- Discussion - was there no result of non-severe thalassemia disease diagnosed in this study? as the prevalence of thalassemia trait is high in Indonesia. Please discuss more.
- Discussion - please discuss more about heavy menstrual bleeding in female in this study particularly ones with low ferritin.

Bukti konfirmasi review

dan

hasil review pertama

ID 4215439

VIEWING AN OLDER VERSION

Anemia among medical students at a private university in Jakarta: Iron deficiency or carrier thalassemia?

Raditya Wratsangka SA CA [1], Endrico Xavieroes Tungka [2], Aditya Krishna Murthi [3], Soegianto Ali [4], Ita Margaretha Nainggolan [5], Edhyana Sahiratmadja [6] + Show Affiliations

Article Type
Research Article

Journal
Anemia

Academic Editor: Duran Canatan

Submitted on: 27 Dec 2023 (5 months ago)

> Abstract

> Author Declaration

> Files 2

Editorial Comments

Duran Canatan AE — 29 JAN 2024

Recommendation
Major Revision Requested

Message for Author
Dear Author, Could you improve your article according to two reviewer's suggestion please? Best regards

Reviewer Reports

Report — Reviewer 2 · 28 Jan 2024

Recommendation
Minor Revision

Report
This manuscript is interesting. However, there are some issues needed to be clarified.
- Methods - please mention more about the cutoff for serum ferritin to diagnose iron deficiency anemia in this study.
- Methods - Since you use BioRad Variant to diagnose thalassemia, please mention why this study use cutoff of Hb A2 >3.5% to diagnose beta-thalassemia trait instead of 4% as suggested with machine
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- Results - could the authors show the level of Hb in this study? as it could affect the symptoms/signs or quality of life in this population.
- Discussion - was there no result of non-severe thalassemia disease diagnosed in this study? as the prevelence of thelassemia trait is high in Indonesia. Please discuss more.
- Discussion - please discuss more about heavy menstrual bleeding in female in this study particularly ones with low ferritin.

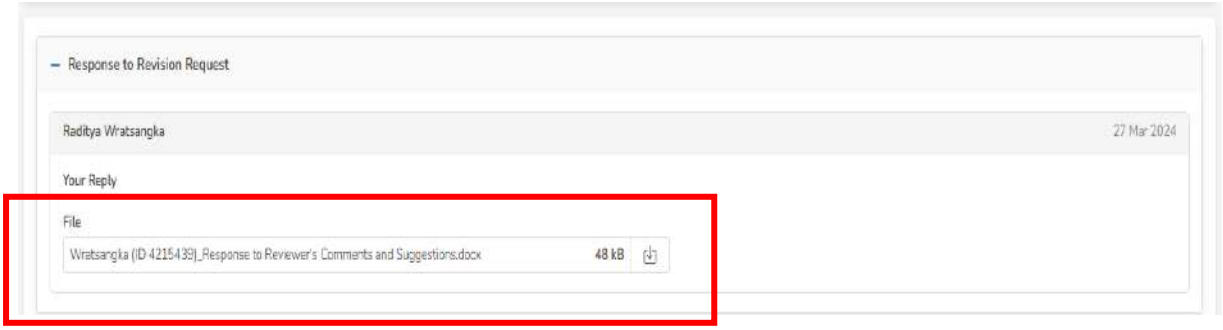
Report — Reviewer 1 · 25 Jan 2024

Recommendation
Major Revision

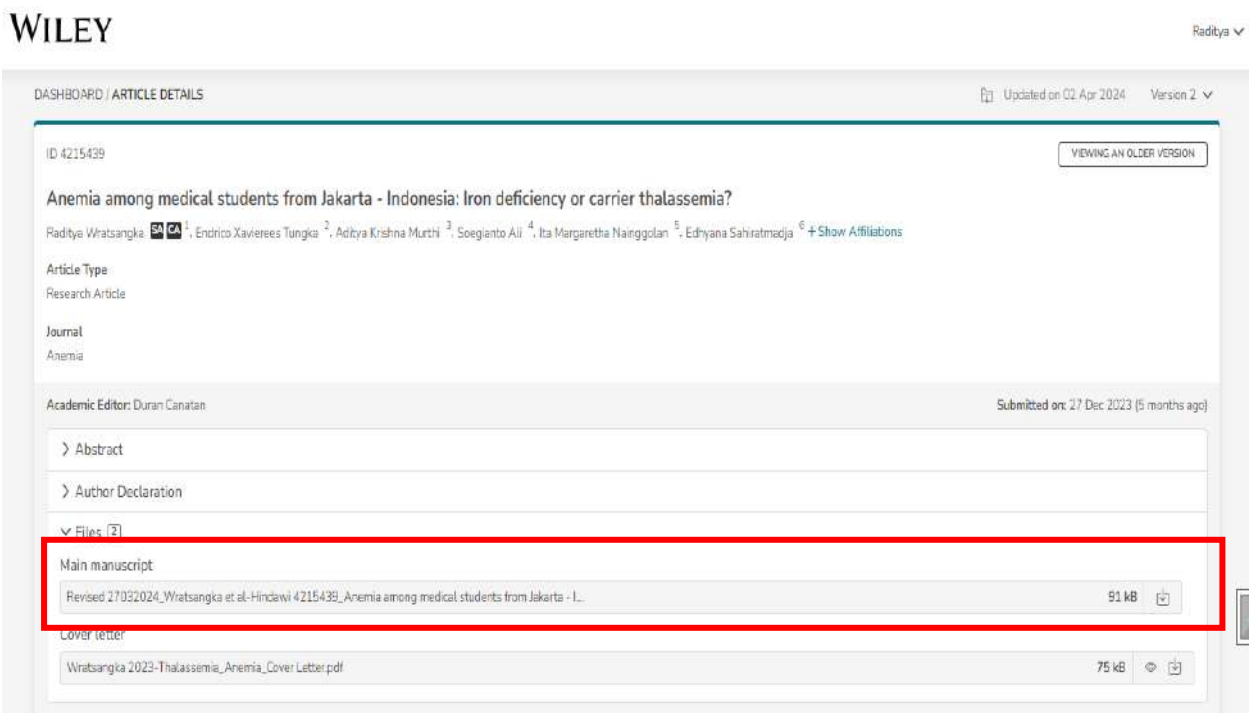
Report
Dear theirs have some suggestions and recommendations to improve the quality of the current manuscript as follows:1- In the study title (Anemiaamong medical students at a private university in Jakarta:Irondeficiency or carrier thalassemia?). It's better to remove the Private university because if the university is private of government it does not matter and it has no any significant impact because already your target are students and those students are the same in both universities if it is private or governmental.2- In study design you mention that (This study was a prospective descriptiveresearch). prospective study (usually you examine something (diseases) before it will happen like monitoring and following the patient till the disease or outcome appears. But here you come and take the sample the anemia already occurs and also mutation already was inherited it seems to be retrospective. Before you make any decision please go and read what is the difference between prospective and retrospective study.Most of people are confusing here and they think that if you meet patient and take sample from them this is prospective but the fact is if you conducted the study before the disease occur this is prospective and if you conducted the disease after this is retrospective. 3- How you select the 140 medical students as your sample size. which the formula used to calculate the sample and this number is less for this study type.4- In your result and limitations i saw the majority of cases are female and we know all parameters related to RBC are less in female when we compare with male . Do these differences not affect your finding? and you need to mention this in your limitations.5- The reference numbers (19) are less for this study. Please cite more references.

Bukti konfirmasi submit revisi pertama, respons kepada reviewer dan artikel yang di resubmit (27 Maret 2024)

Respons to Reviewer's Comments and Suggestion



Re-submit revised article



Response to the reviewers regarding article entitled "Anemia among medical students at a private university in Jakarta: Iron deficiency or carrier thalassemia?"

Manuscript ID: 4215439

A. Reviewer 1 Comments to the Author

1- *In the study title (Anemia among medical students at a private university in Jakarta: Iron deficiency or carrier thalassemia?). It's better to remove the Private university because if the university is private of government it does not matter and it has no any significant impact because already your target are students and those students are the same in both universities if it is private or governmental.*

Response:
Thank you for your suggestion. We have changed the title as suggested.

Previous title:
Anemia among medical students at a private university in Jakarta: Iron deficiency or carrier thalassemia

New title:
Anemia among medical students from Jakarta - Indonesia: Iron deficiency or carrier thalassemia?

Notes:
We initially specifically referred to "private university" because it was associated with much more expensive education costs at private universities compared to state or government universities in Indonesia, so students at a private university are estimated to have a good socio-economic and nutritional status and are less likely to suffer from nutrient or iron (Fe) deficiency anemia.

2- *In study design you mention that this study was a prospective descriptive research. Prospective study usually you examine something (diseases) before it will happen, like monitoring and following the patient till the disease or outcome appears. But here you come and take the sample the anemia already occurs and also mutation already was inherited it seems to be retrospective. Before you make any decision please go and read what is the difference between prospective and retrospective study. Most of people are confusing here and they think that if you meet patient and take sample from them this is prospective but the fact is if you conducted the study before the disease occur this is prospective and if you conducted the disease after this is retrospective.*

Response:
Thank you very much for your valuable insight on our study design. We have corrected the study designed in the method section.

… This study was **descriptive research** with a cross-sectional approach to explore the anemia prevalence among medical students and the etiology of the anemia… ***(page 4)***

3- *How you select the 140 medical students as your sample size. which the formula used to calculate the sample and this number is less for this study type.*

Response:
We appreciate your thought of our sample size.

This study is a continuation (phase II) of another previous study (phase I) that examined the knowledge, experience, and attitudes of medical students about thalassemia and their perception of quality of life. These two studies were conducted consecutively at almost the same time during the COVID-19 pandemic (May - June 2021) on the same research subjects. The participants were recruited from 1st to 4th year medical students (class of 2016 - 2020) from one of the faculties of medicine in Jakarta - Indonesia. Recruitment of participants begins with socialization activities conducted by the research team directly for all students, both online and offline. The online session was held on May 24, 2021, and continued with consecutive offline sessions on June 23 - 29, 2021. In phase I of this study, participants were selected from Universitas Trisakti medical students who voluntarily registered after research socialization. During the socialization, officers also conveyed the need to take blood samples from all participants for carrier screening in phase II research. These sessions allow dialogue between participants and members of the research team about research objectives, methodologies, and data collection processes. Those who can participate in this study must be medical students who are still actively studying, domiciled in Jakarta, not have a chronic illness and/or have not had a blood transfusion within 3 months before the blood draw, and appear to be healthy during data collection.
A formula for estimating a single proportion of the participation rate is applied to calculate the sample size. With a total of 619 students from the class of 2016-2020, limited population correction (FPC) was applied to adjust the final sample size. The sample size calculation formula with FPC is:

$$n = \frac{\frac{Z^2 . p(1-p)}{e^2}}{1 + (\frac{Z^2 . p(1-p)}{e^2 N})}$$

To estimate 90% of the participation rate, with a confidence level of 95% ($Z\alpha/_2 = 1.96$) and a margin of error of 5% (E), the minimum sample size required after correction is 113 participants. Of the 375 students who participated in the socialization session, as many as 140 of them decided to participate in the research. The selection of subjects as samples was carried out by consecutive non-random sampling.

Knowledge, attitudes, and experience related to thalassemia; perception of quality of life; socio-demographic status; and participants' economic status were assessed using a series of online questionnaires (Google Form) as phase I. Seven milliliters of venous blood from each of the same people who took part in the study were also collected in two three-milliliter EDTA tubes so that thalassemia carriers could be screened three times in phase II.

4- *In your result and limitations I saw the majority of cases are female and we know all parameters related to RBC are less in female when we compare with male . Do these differences not affect your finding? and you need to mention this in your limitations.*

Response:
Your concern has been taken into our consideration.

Due to limited number of respondents, we have pooled the value together. Indeed, there are parameters related to RBC which are less in female compared to male. We have added this limitation in the text. …. ***(page 13 and 16)***

5- *The reference numbers (19) are less for this study. Please cite more references.*

Response:
Thank you very much for your suggestion to add more references.

B. ***Reviewer 2 Comments to the Author***

B.1. Methods

1. *Please mention more about the cutoff for serum ferritin to diagnose iron deficiency anemia in this study.*

Response:
Ferritin measurement was analyzed using the chemiluminescent magnetic microparticle immunoassay (CMIA) method (Architect® i2000) by Prodia Clinical Laboratory. Their reference normal value of ferritin level was 11 – 148 ng/mL.

Reference:
Reference intervals established using indirect method for serum ferritin assayed on Abbott Architect i2000SR analyzer in Chinese adults.
(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7083431/pdf/JCLA-34-e23083.pdf)

Level of serum ferritin of the study (ng/mL)

Gender		N	Minimum	Maximum	Mean	Std. Deviation
Female	Ferritin	97	1.83	287.09	56.5455	59.10454
	Valid N (listwise)	97				
Male	Ferritin	43	27.81	440.47	169.2214	101.84673
	Valid N (listwise)	43				

Notes:
Serum ferritin is a blood test used to measure the amount of iron stored in the body. It is one of the most important tests for diagnosing iron deficiency anemia. Ferritin levels can give a good indication of the body's total iron stores, although levels can also be elevated in cases of inflammation or chronic disease, as ferritin is an acute-phase reactant.

For adults, the typical reference ranges for serum ferritin are:
- **Men**: 20-250 ng/mL
- **Women**: 10-120 ng/mL

However, these ranges can vary slightly depending on the laboratory.
In the context of iron deficiency anemia:
- **Ferritin levels below 15-30 ng/mL** are generally considered diagnostic of iron deficiency anemia, indicating depleted iron stores.
- **Levels between 30-100 ng/mL** might suggest iron deficiency depending on the presence of other indicators of iron deficiency anemia (like a low hemoglobin

concentration, low mean corpuscular volume, or low serum iron) and the clinical context, especially in the setting of chronic diseases.

It's important to interpret serum ferritin levels in conjunction with other tests and clinical findings because various factors can influence ferritin levels. For instance, in conditions with chronic inflammation, infection, liver disease, or malignancies, ferritin levels might be elevated regardless of iron stores, potentially masking iron deficiency. In such cases, other iron tests such as serum iron, total iron-binding capacity (TIBC), and transferrin saturation might be considered alongside ferritin to get a clearer picture of iron status.

2. *Since you use BioRad Variant to diagnose thalassemia, please mention why this study use cutoff of Hb A2 >3.5% to diagnose beta-thalassemia trait instead of 4% as suggested with machine*

Response:
Indeed, the machine BioRad Variant has suggested cut off value of 4% to diagnose beta-thalassemia trait. Here's an explanation of why we're using lower cutoff (HbA_2 >3.5%):

The cutoff value of hemoglobin A_2 (HbA_2) to diagnose beta-thalassemia trait can vary slightly depending on the laboratory and the population being studied. However, in general, a level of HbA_2 greater than 3.5% is often considered suggestive of beta-thalassemia trait. This is typically confirmed through additional testing, such as hemoglobin electrophoresis or genetic testing, to definitively diagnose beta-thalassemia trait. It's important to note that interpretation of HbA_2 levels should be done in conjunction with clinical findings and other laboratory tests for a comprehensive diagnosis.

We have added this information in …… (page 11-12)

3. *The regular cutoff for diagnose Hb E by BioRad Variant is Hb A 25-35%, if Hb A is between 10-25%, it usually means that this patient have both alpha-thalassemia (trait/disease) with Hb E trait. Please discuss more particularly with the cutoff of Hb A_2 at >13%*

Response:
We apologize we do not understand for this comment about '… HbA being 25-35%, if Hb A_2 is between 10-25%.....'

In our different study, we have also performed Hb variant using capillary Hb Electrophoresis (Sebia), and we see that the HbA_2 is normal and there is a peak of HbE.

Indeed, that could be diagnosed as Hb E trait and also possible alpha-thalassemia disease, something that we should be aware of. Since we did not examine for alpha-thalassemia deletion and/or mutation, this can not be well concluded.

We have put this in the limitation …… (*page 12*)

References:

Challenges in Thalassemia Carrier Detection in a Low Resource Setting Area of Eastern Indonesia: The Use of Erythrocyte Indices
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7813278/
https://doi.org/10.4084/MJHID.2021.003

Notes: :
To distinguish between ***Hemoglobin E (HbE) trait*** and ***alpha thalassemia trait***, one must comprehend the genetic and hematological distinctions that exist between these two disorders. Both are types of thalassemia, hereditary blood illnesses characterised by decreased hemoglobin production, resulting in anemia. Nevertheless, they vary in terms of the particular globin chains that are impacted and their geographical distribution.

A. Genetic Variations:

1) **HbE Trait**: HbE arises from a mutation occurring in the beta-globin gene. Individuals with HbE characteristic possess a mutation in one of their beta-globin genes, while the other gene remains unaffected. This leads to the synthesis of an atypical variant of hemoglobin, known as Hemoglobin E, in addition to the regular hemoglobin.
2) **Alpha Thalassemia Trait**: Alpha thalassemia is a genetic condition caused by the removal or alteration of the alpha-globin genes. The usual number of alpha-globin genes in humans is four. Individuals with alpha thalassemia trait experience a deletion or mutation of one or two of these genes, resulting in a decrease in the formation of alpha-globin chains.

B. Haematological observations:

1) **HbE Trait**: Individuals with HbE trait commonly exhibit mild microcytic anemia, characterised by smaller-than-normal red blood cells. Hemoglobin electrophoresis, a blood test, detects the existence of Hemoglobin E along with the usual hemoglobin A.
2) **Alpha Thalassemia Trait**: Alpha thalassemia trait refers to a condition where one or two alpha-globin genes are damaged. Individuals with this condition may experience mild anaemia or have normal hemoglobin levels, but with the presence of microcytosis, which is characterised by minute red blood cells. Hemoglobin electrophoresis may not provide significant assistance in identifying alpha thalassemia trait, and a conclusive diagnosis frequently necessitates genetic testing.

C. Clinical Presentation:

1) **HbE Trait**: The majority of individuals with HbE trait do not show any symptoms and are typically identified by chance during regular blood tests.
2) A**lpha Thalassemia Trait**: Individuals with one or two gene deletions (alpha thalassemia minor or trait) typically do not show symptoms or experience only mild anaemia.

D. Geographical distribution:

1) **HbE Trait** exhibits a high prevalence in Southeast Asia, specifically in countries such as Thailand, Laos, Cambodia, as well as some regions of India and Bangladesh.
2) **Alpha Thalassemia Trait** is prevalent in populations originating from the Mediterranean region, the Middle East, Africa, Southeast Asia, and among individuals of these lineages.

In order to definitively distinguish between HbE trait and alpha thalassemia trait, medical professionals frequently utilise a combination of haematological tests, including a complete blood count (CBC), hemoglobin electrophoresis, and occasionally DNA analysis to identify genetic abnormalities or deletions. Individuals who are suspected of having these disorders should seek consultation with a healthcare professional or a hematologist in order to obtain an accurate diagnosis and appropriate management.

B.2. Results

1. *Patients with anemia but have normal MCV/MCH, what could be the cause? Please discuss more.*

Response:
Thank you for your input.

We are not having patients in our study, but respondents who are medical students. As we consider them to be in good condition, the explanation of the etiology of students with anemia who have normal MCV or MCH could be as follows:

Anemia characterized by a normal mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) is known as normocytic or normochromic anemia. This form of anemia is distinguished by red blood cells that are of average size (normocytic) and contain a normal amount of hemoglobin (normochromic). The causes can vary and encompass:

1) Acute Blood Loss: The abrupt loss of blood caused by trauma, surgery, or internal bleeding can result in anemia because the body does not have sufficient time to compensate for the depletion of red blood cells.
2) Chronic diseases such as persistent infections, inflammatory conditions (such as rheumatoid arthritis or lupus), and chronic kidney disease can result in the development of anemia. Inflammation or a pathological process can disrupt the synthesis of erythrocytes or reduce their longevity.

3) Aplastic anemia is a disorder when the bone marrow doesn't make enough red blood cells. It can be due to viral infections, autoimmune illnesses, exposure to hazardous substances, or certain drugs.
4) Hemolytic anemias: These are conditions where red blood cells are destroyed quicker than they can be created. Causes can include autoimmune illnesses, some infections, and responses to medications or poisons.
5) Anemia of persistent disease: a prevalent kind of anemia, particularly in hospitalized patients, sometimes associated with persistent infection, inflammation, or malignancy. It's assumed to be caused by the body's response to the chronic disease, which includes the retention of iron in the macrophages and a decrease in erythropoiesis (the creation of red blood cells).
6) Renal Anemia: Chronic renal disease can lead to anemia by lowering the production of erythropoietin, a hormone needed for red blood cell development.
7) Bone Marrow Disorders: Conditions such as myelodysplastic syndromes, which disrupt the bone marrow's ability to create blood cells, can lead to normocytic or normochromic anemia.
8) Endocrine problems: Certain endocrine problems, such as hypothyroidism or hypopituitarism, can contribute to anemia by disrupting the production of hormones that regulate red blood cell development.

Diagnosis of the specific cause of normocytic, normochromic anemia involves a thorough medical history, physical examination, and a series of laboratory tests beyond just the complete blood count (CBC) that measures MCV and MCH, such as reticulocyte count, serum iron levels, ferritin, total iron-binding capacity (TIBC), vitamin B12, and folate levels, as well as tests for renal function and possible bone marrow examination. (*page 10*)

2. *Patients who have low MCV/MCH but normal ferritin and DNA for alpha- and beta-globin genes, what could be the cause? Please discuss more.*

Response:
We are not having patients in our study, but respondents who are medical students. As we consider them to be in good condition, the etiology of students who have low MCV/MCH but normal ferritin and DNA for alpha- and beta-globin genes can be explained as follows:

Patients with low mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values typically exhibit signs of microcytic and hypochromic anemias, which are often associated with iron deficiency or disorders of hemoglobin synthesis like thalassemia. However, if ferritin levels are normal and there are no mutations in the DNA for alpha- and beta-globin genes (which would indicate thalassemia), other potential causes could include:

1) Anemia of chronic disease: This type of anemia can present with low MCV and MCH values and is often associated with chronic infections, autoimmune diseases, or chronic inflammatory conditions. It is characterized by impaired iron utilization despite normal or increased iron stores, as indicated by normal ferritin levels.

2) Lead poisoning: Chronic exposure to lead can lead to a microcytic anemia similar to that seen in iron deficiency or thalassemia, but with normal ferritin levels.
3) Sideroblastic anemia: This group of disorders is characterized by the presence of ringed sideroblasts in the bone marrow and can present with normal or elevated ferritin levels. Sideroblastic anemias can be inherited or acquired and involve impaired heme synthesis within the red blood cells.
4) Copper deficiency: Copper is a cofactor for enzymes involved in iron metabolism and red blood cell formation. A deficiency in copper can lead to microcytic anemia that may mimic iron deficiency anemia but occurs in the context of normal iron stores.
5) Rare hemoglobin variants: Although the most common hemoglobinopathies are related to alpha- and beta-globin genes, there are rare variants that might not be detected in standard screening tests for these genes.
6) Chronic kidney disease: In some cases, chronic kidney disease can lead to a normochromic, normocytic anemia, but it may initially present as microcytic and hypochromic. The anemia is primarily due to reduced erythropoietin production but can be compounded by other factors such as chronic inflammation.

It's important to note that the diagnosis of anemia and its underlying cause is complex and requires a comprehensive approach, including detailed patient history, physical examination, and a broader panel of laboratory tests beyond MCV, MCH, and ferritin levels. Consulting with a hematologist may be beneficial for patients with unexplained anemia. (*page 10)*

3. *Could the authors show the level of Hb in this study? As it could affect the symptoms/signs or quality of life in this population.*

Response:
The level of Hb in this study is **12.7 g/dL** among female and **15.7 g/dL** among male students. In general, the quality of life in this population is good. However, further classification based on WHO, the anemia was found in **13.6% (n=19)** female and **0%** male students.

Level of Hemoglobin of the study (g/dL)

Gender		N	Minimum	Maximum	Mean	Std. Deviation
Female	Hemoglobin	97	9.50	16.00	12.7485	1.11384
	Valid N (listwise)	97				
Male	Hemoglobin	43	14.00	17.90	15.7047	.95891
	Valid N (listwise)	43				

If a female is experiencing mild anemia but is otherwise in good physical condition, it's essential to consider the potential causes and appropriate management strategies for her condition. Anemia, characterized by a decrease in the number of red blood

cells or the amount of hemoglobin in the blood, can lead to various symptoms, including fatigue, weakness, pale skin, and shortness of breath, among others. However, mild cases may have minimal or no symptoms, especially if the individual is generally healthy and physically active. ... (*page 9*)

B.3. Discussion

1. *Was there no result of non-severe thalassemia disease diagnosed in this study? as the prevalence of thalassemia trait is high in Indonesia. Please discuss more.*

Response:
This study has explored the trait status among medical students. The prevalence of thalassemia trait in Indonesia is considered moderate (6-10%) in some area to 15 in other area, however, no result of non-severe thalassemia disease has been found.
…. (page 11)

2. *Please discuss more about heavy menstrual bleeding in female in this study particularly ones with low ferritin.*

Response:
Another limitation of this study is that we did not ask in detail about the pattern of menstrual bleeding, whether it is heavy bleeding, therefore, we could not analyse the relation between menstrual bleeding and low ferritin. We suggest in the limitation section to add this information in the future study.

Notes:

The correlation between menstruation and low ferritin levels is highly significant, as menstruation is a prevalent factor in causing iron insufficiency, resulting in decreased ferritin levels in women who are of reproductive age. Ferritin is a hemoprotein found in blood cells that serves as a marker for the overall iron reserves in the body. During menstruation, women experience blood loss, resulting in the depletion of iron levels. In the absence of dietary intake or supplementation, this depletion might result in iron insufficiency.
Iron deficiency is a prevalent dietary shortage that occurs globally and can result in anemia. Anemia is a condition characterized by a reduction in the quantity of red blood cells or hemoglobin in the blood, causing symptoms such as weariness, weakness, and difficulty breathing. Iron is essential for the production of hemoglobin, which is responsible for transporting oxygen to the body's tissues. Therefore, a lack of iron directly affects the body's ability to carry oxygen.

Women who experience heavy menstrual flow, also known as menorrhagia, have a significantly increased likelihood of developing iron deficiency anemia. In these instances, the quantity of blood and iron lost throughout each menstrual cycle surpasses the body's capacity to replenish it, resulting in gradually declining ferritin levels. Women who experience regular menstrual flow might still develop iron

insufficiency if they do not consume enough iron in their diet or if they experience other forms of iron loss, such as gastrointestinal hemorrhage.

In order to treat or prevent iron shortages caused by menstruation, it is crucial for women to ensure they consume enough iron in their diet. Iron-rich foods encompass red meat, chicken, fish, lentils, beans, and cereals fortified with iron. Iron supplements may be prescribed, particularly for women with excessive monthly bleeding or those diagnosed with low ferritin levels or iron deficiency anemia. Nevertheless, it is important to take iron supplements with the supervision of a healthcare professional, since an excessive amount of iron can result in toxicity and various health complications.
To summarize, there is a clear correlation between menstruation and low ferritin levels as a result of iron depletion from menstrual bleeding. Sufficient consumption of iron-rich foods and, in certain instances, the use of supplements are crucial for the prevention and treatment of this disorder. *(page 13-14)*

Resubmit Revised Article (27 Maret 2024)

Anemia among medical students from Jakarta - Indonesia:

Iron deficiency or carrier thalassemia?

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ABSTRACT

BACKGROUND: Anemia, a global health concern, affects one-fourth of the global population, particularly women. In Indonesia, its prevalent is at 23.7%, with 32.0% among 15–24 year-olds. Factors include poor nutrition, infectious diseases, chronic diseases, inherited disorders, and inadequate healthcare access. This study aimed to investigate anemia prevalence and its etiology among medical students from Jakarta.
METHODS: This study was a descriptive research with a cross-sectional approach. Undergraduate students aged 18-23 years old were selected and consented to participate by a consecutive non-random sampling methods. Laboratory blood data were evaluated (including Hb, MCV, MCH, HbA_2, and Ferritin levels) and DNA was isolated to confirm the type of thalassemia carrier.
RESULTS: In total, 140 medical students, mainly female, were recruited. Anemia was found in 13.6% (11.4% had low MCV and/or MCH), and 16.5% had low MCV and/or MCH without anemia. Hb electrophoresis revealed high HbA_2 values, suggesting the HbE variant (2.1%), and β-thalassemia carrier (0.7%). DNA analysis confirmed *the cd26 mutation* and *heterozygous IVS1nt5*. Among those without anemia, 5% had *α-deletion*, while in the group with anemia, 1.4% had *α-deletion* (with coexistent IDA), 3.6% had *α-deletion*, and 0.7% had *β-mutation*.
CONCLUSION: DNA analysis can identify specific mutations associated with alpha-thalassemia, distinguishing between iron deficiency anemia and the alpha-thalassemia trait. Thalassemia screening should involve low MCV and/or MCH values as the first step (stage 1), followed by Hb analysis (stage 2) and DNA analysis (stage 3). In common areas, a combination of Hb and DNA testing is best. However, healthcare professionals must diagnose and treat thalassemia, as proper management relies on accurately identifying the underlying condition.

INTRODUCTION

Anemia is a worldwide public health issue, affecting both developed and developing nations. Anemia is also the third leading cause of years with lived disability (YLDs).[1] One-fourth of the global population is estimated to be anemic, with cases increasing rapidly in women. Over half a billion women between the ages of 15 and 49 make up the global anemia prevalence in 2019, including women of reproductive age (WRA; 29.9%), pregnant women (36.5%) and non-pregnant WRA (29.6%), with sub-Saharan Africa and South Asia having the most significant rates.[1] In 2021, there is an increase of 420 million cases over three decades.[1,2] Data in Indonesia revealed that the prevalence of anemia in Indonesia was 23.7%.[3] Additionally, the prevalence of anemia among individuals aged 15-24 years was 32.0%.

The high prevalence of anemia among young adults in Indonesia may differ among regions and population subgroups and is likely due to a combination of factors, including poor nutrition, infectious diseases, chronic diseases, inherited disorders (sickle cell anemia, thalassemia), and inadequate access to healthcare.[4,5] Interestingly, recent evidence has shown that the awareness programs and laboratory examinations have identified more thalassemia carriers, minors, or traits among young adults.[6]

Thalassemia carrier has a small red blood size (microcytic) as shown in low MCV values and/or less red blood cell color (hypochromic) as shown in low MCH values. Thalassemia carrier does not necessarily have low Hb. Anemia and thalassemia can cause low levels of hemoglobin in the blood, leading to low levels of red blood cells and decreased oxygen delivery to tissues.[7] Thalassemia is one condition that can cause anemia, however, thalassemia minor can be found without anemia.[7] The two conditions share some similarities, but have some essential differences.

Thalassemia is an inherited autosomal recessive gene disorder caused by a decreased or absent production of one or two types of globin chains, and classified according to the particular globin chain affected, such as α-globin and β-globin. Thalassemia is also a significant health issue in Indonesia, particularly among young adults.[8,9] Indonesia is located along the 'Thalassemia Belt', a geographic region that stretches from the Mediterranean to Southeast Asia, where the prevalence of thalassemia is highest.[10–12]

Medical students, as part of the young adult population, are obliged to study various diseases, including anemia and thalassemia in their courses on genetics, hematology, and other related topics. Medical students' knowledge, attitudes, and practice towards thalassemia are likely to be shaped by various factors, including their academic and personal backgrounds, their exposure to patients with thalassemia, and their understanding of the social and cultural contexts in which the disease occurs. Medical students who look physically fit may not even know their status of anemia and carriers of thalassemia traits.[13–16] Study in Malaysia involving 400 undergraduate medical students has reported that 14.5% students had hypochromic microcytic red cell indices, 11% showed thalassemia red cell indices, and only 3.5% had iron deficiency (IDA) red cell index which were finally confirmed by serum iron/TIBC analysis.[17]

Therefore, it is interesting to also explore the etiology of anemia among students in Indonesia compared to the neighboring country Malaysia. The study aimed to explore the anemia prevalence and etiology among medical students from Jakarta.

MATERIAL AND METHOD

Study design

This study was descriptive research with a cross-sectional approach to explore the anemia prevalence among medical students and the etiology of the anemia. Laboratory blood data were evaluated (including Hb, MCV, MCH, HbA_2, and Ferritin levels) and DNA was isolated to

confirm the type of thalassemia carrier (trait). The study was approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Trisakti (No. 011/KER/FK/IV/2021).

Subjects

Undergraduate students of the Faculty of Medicine, Universitas Trisakti, aged 18-23 were selected and consented to participate by a consecutive non-random sampling methods. Those who had a history of chronic disease and had received blood transfusions in the past three months were excluded from this study.

Complete blood count and HbA_2 measurement

Venous blood sampling was collected in a 3 ml EDTA tube to be checked further for a complete blood count (CBC), followed by Ferritin measurement and HbA_2 determination by High Performance Liquid Chromatography (Bio-Rad). CBC was analyzed using the Flow Cytometry Method Using a Semiconductor Laser (Sysmex-XN®); ferritin measurement was analyzed using the chemiluminescent magnetic microparticle immunoassay (CMIA) method (*Architect® i2000*). The definition of anemia is according to WHO standards based on sex (men <13g/dL and women <12g/dL). The red blood cell (RBC) indices were then analyzed based on a guide from the Ministry of Health, Republic of Indonesia, MCV< 80 fL and MCH < 27pg values which were used as initial thalassemia screening. Furthermore, Hb analysis data were classified based on HbA_2 fraction values according to the protocol of the manufacturer (Bio-Rad) where < 2.0% suggested for α-thalassemia carriers; 2.0–3.5% as normal value; >3.5% was indicated as β-thalassemia carriers; and very high HbA_2 level peak shown indicated HbE carriers. Hereafter, subjects who had normal HbA_2 but had low/normal Hb and/or low MCV and/or low MCH were examined further for DNA analysis. Those with low MCV and/or MCH and with normal HbA2 value (2-3.5%) were further confirmed with DNA examination to distinguish the etiology of anemia whether due to iron deficiency or alpha thalassemia carrier. The students

were also checked for their ferritin levels to determine whether anemia was due to iron deficiency

DNA genotyping identification

Subjects with low MCV and/or MCH, low and normal Hb, and low and normal ferritin were identified by the level of HbA2 and subsequently confirmed their genotype by DNA analysis (N = 39). Due to the limited availability of examination kits, DNA tests were carried out on 30 of 39 subjects, whose selection was done by simple random sampling.

We used the α-Globin StripAssay® (Ref. 4-160, ViennaLab Diagnostics GmbH) to detect 21 common α-globin mutations in separated teststrips. The procedure includes three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers, (3) hybridization of amplification products to teststrips containing allele-specific oligonucleotide probes immobilized as an array of parallel line. The bound biotinylated sequences on specific wild types and mutants are detected using streptavidin-alkaline phosphatase and color substrates. We prepared three separate PCR mixtures (A1, A2, and B), following both PCR conditions and hybridization according to the manufacturer's instructions. We analyzed the hybridization results by comparing the line with the provided CollectorTM sheet. In heterozygous conditions, both mutated bands and normal bands will appear. In homozygous mutations, only the positive band is colored. If no mutations are contained in the StripAssay panel, only PCR and normal controls are colored. Furthermore, to identify β-globin gene mutations, we used the β-Globin StripAssay® SEA (Ref. 4-150, Vienna Lab Diagnostics GmbH). The assay covers 22 common β-globin mutations in one strip only. Unlike the assay for α-thalassemia, the assay for β-thalassemia was only in one PCR mixture and followed the manufacturer's instructions. The hybridization procedure and analysis of test strips results were the same as in α-globin gene mutation detection.

RESULT

In total, 140 medical students were recruited, primarily female (69.3%; n=97), with a median age of 20 (range 18–26 years). The level of Hb in this study is 12.7 g/dL among female and 15.7 g/dL among male students. Anemia was found in 13.6% (n=19) of the female students (table 1). Interestingly, 16.5% of students (n=23; 18 females and 5 males) were not anemic but had low MCV and/or low MCH. These students were subjected to the next level of the thalassemia screening program as indicated by the Ministry of Health. In addition, 16 (11.4%) students who were anemic and had low MCV and/or MCH were examined further to determine the etiology of anemia.

Table (1). Hemoglobin level based on gender and MCV and/or MCH level in medical students (n=140)

	Level of Hemoglobin		
	Not Anemia n (%)	Anemia n (%)	Total n (%)
Male	43 (30.7)	0	43 (30.7)
MCV and/or MCH			
• Normal	38 (27.1)	0	38 (27.1)
• Low	5 (3.6)*	0	5 (3.6)
Female	78 (55.7)	19 (13.6)	97 (69.3)
MCV and/or MCH			
• Normal	60 (42.9)	3 (2.1)	63 (45.0)
• Low	18 (12.9)*	16 (11.4)**	34 (24.3)

Note: Anemia Hb: <13.0 g/dL (male); < 12.0 g/dL (female)
Low MCV: <80 fL
Low MCH: <27 pg

* Not anemia but with low MCV and/or MCH, subjected for further examination

** Anemia and were further examined to explore the etiology of anemia

In our study, the students were examined by Hb electrophoresis (level 2), resulting in a very high HbA2 value (>13%), suggesting an HbE variant (2,1%; n=3); and a high HbA2 value (>4% to 13%), indicating β-thalassemia carrier (0.7%; n=1); that was confirmed by DNA analysis (level 3), resulting in cd26 mutation and heterozygous IVS1nt5, respectively (table 2).

Table (2). Complete blood count examination (Level 1) followed by Hb variant A2 (Level 2) and DNA confirmation (Level 3) among medical students from a private university in Jakarta (n=140)

Level 1		Level 2		
MCV and/or MCH	Hb level	HbA2		
		2 – 3.5% n (%)	> 3.5% n (%)	>>> 13% n (%)
Low (n=39)	Not Anemia	20 (14.3) ***	0	3 (2.1) *
	Anemia	15 (10.7) ****	1 (0.7) **	0
Normal (n=101)	Not Anemia	98 (70.0)	0	0
	Anemia	3 (2.1)	0	0

Note:
*Cd26 (G>A) heterozygote;
**IVS1nt5 heterozygote;
***Further DNA analysis needed whether iron deficiency anemia or alpha Thalassemia carrier
****Need iron therapy first

Among those without anemia (n=15), 5% (n=7) had α-deletion (table 3). Furthermore, in a group with anemia (n=14), α-deletion (with coexistent IDA) was detected in 1.4% (n=2), αdeletion 3.6% (n=5), and β-mutation in 0.7% (n=1).

Table (3). Evaluation of Ferritin among students (n=30) with low MCV and/or MCH (Level 1) in relation to Hemoglobin level, HbA2 (Level 2) and DNA genotype (Level 3)

Level 1	Level 2	Level 3

(Low MCV/MCH) N = 30	Hb Electrophoresis (Hb A_2)			DNA Genotype
	2-3.5	> 3.5	>>>	
• **Not Anemia** (n = 16)				
• Ferritin Low (n = 1)	1			Beta (β): IVS1-nt5 [G>C] heterozygote + coexistent IDA ***)
			3	Confirmed HbE: codon 26 [G>A] heterozygote
• Ferritin Normal (n = 15) *)	4			No alpha (α) deletion or mutation found, need further exploration
	4			Alpha (α): Single α globin gene deletion 3.7 kb type heterozygote
	3			Alpha (α): Double α globin gene deletion SEA type heterozygote
• **Anemia** (n = 14)				
	4			No-α deletion or mutation; suggesting IDA
• Ferritin Low (n = 6)	1			Alpha (α): 3.7 kb triplicated α gene heterozygote + coexistent IDA ***)
	1			Alpha (α): α2 cd 59 [GGC>GAC] heterozygote + coexistent IDA ***)
	3			Alpha (α): Double α globin gene deletion SEA type heterozygote
• Ferritin Normal (n = 8) **)	1			Alpha (α): α2 cd 142 [TAA>CAA] (Hb Constant Spring) heterozygote
	1			Alpha (α): α2 cd 59 [GGC>GAC] heterozygote
		1		Beta (β): IVS1-nt5 [G>C] heterozygote

Note. Anemia was designated as Hb < 12.0 g/dL for females and Hb < 13.0 g/dL for males
*) One sample DNA examination failed
**) Two samples DNA examination failed
***) Point of interest; IDA with coexistence confirmed Alpha (ά)

DISCUSSION

Our study has shown that among medical students aged 18 to 23 years old, anemia has been detected in 13.6% (n=19) students, all females. Interestingly, 16.5% of students (18 females and 5 males) who are not anemic, have low MCV and/or MCH. All these students have been examined for ferritin, Hb electrophoresis, and DNA genotyping for alpha and beta-globin to explore the etiology of anemia.

If a female is experiencing mild anemia but is otherwise in good physical condition, it's essential to consider the potential causes and appropriate management strategies for her condition. Anemia, characterized by a decrease in the number of red blood cells or the amount of hemoglobin in the blood, can lead to various symptoms, including fatigue, weakness, pale skin, and shortness of breath, among others. However, mild cases may have minimal or no symptoms, especially if the individual is generally healthy and physically active.[18]

Medical students who have a middle-class socio-economic status and are knowledgeable about anemia and thalassemia should be aware of the effects of poor nutrition and inadequate dietary iron intake. Not consuming enough iron-rich foods (including red meat, poultry, fish, beans, lentils, tofu, fortified cereals, and green leafy vegetables) in their diet can lead to IDA, which is assumed to be low.[19–21] However, the results showed that 13.6% (n=19) had anemia and additionally, 16.5% (n=23) were not anemic but had low MCV/MCH.

Anemia characterized by a normal MCV/MCH is known as normocytic or normochromic anemia. This form of anemia is distinguished by red blood cells that are of average size (normocytic) and contain a normal amount of hemoglobin (normochromic). The causes can vary and encompass: acute blood lost, chronic diseases, aplastic anemia, hemolytic anemias, anemia of persistent disease, renal anemia, bone marrow disorders, and endocrine problems such as hypothyroidism or hypopituitarism. Diagnosis of the specific cause of normocytic, normochromic anemia involves a thorough medical history, physical examination, and a series of laboratory tests beyond just the complete blood count (CBC) that measures MCV and MCH, such as reticulocyte count, serum iron levels, ferritin, total iron-binding capacity (TIBC), vitamin B12, and folate levels, as well as tests for renal function and possible bone marrow examination.

Patients with low MCV/MCH values typically exhibit signs of microcytic and hypochromic anemias, which are often associated with iron deficiency or disorders of hemoglobin synthesis like thalassemia. However, if ferritin levels are normal and there are no mutations in the DNA for alpha- and beta-globin genes (which would indicate thalassemia), other potential causes could include: anemia of chronic disease, lead poisoning, sideroblastic anemia, copper deficiency, rare hemoglobin variants, and chronic kidney disease. It's important to note that the diagnosis of anemia and its underlying cause is complex and requires a comprehensive approach, including detailed patient history, physical examination, and a

broader panel of laboratory tests beyond MCV, MCH, and ferritin levels. Consulting with a hematologist may be beneficial for patients with unexplained anemia.

Indonesia, located in the thalassemia belt area, has many thalassemia careers in Southeast Asia, making anemia in young adulthood a significant concern. However, no result of non-severe thalassemia disease has been found among medical students in this study. Anemia in young adults can have other causes, such as hemoglobin abnormalities or hemoglobinopathy, as the most common causes. Efforts to detect early thalassemia careers have not been carried out systematically. Around 3.0-10.0% of the Indonesian population carry β-thalassemia (β-thal) and 2.6-11.0% of the population carry α-thalassemia (α-thal), with the highest incidence rates reported in regions such as Bali, East Nusa Tenggara, and Maluku.[22] It is estimated that around 2500 babies are born with β-thal major (β-TM) each year.[22].

Iron Deficiency Anemia or Thalassemia Carrier?

Further analysis with Hb electrophoresis has shown that it is difficult to distinguish between IDA and alpha-thalassemia carriers. Both conditions can lead to microcytic (small red blood cells) and hypochromic (pale) red blood cells, making it necessary to perform additional tests, such as DNA examination, to make a definitive diagnosis. In restricted facilities, MCV<80 fl and/or MCH<27 pg is the optimal first-round mass screening approach for β-thalassemia carriers. Gradually implement Hb electrophoresis, and DNA analysis should be done in many regions to confirm mutations for accurate carrier detection wherever possible.[23] Since the machine BioRad Variant that we used has suggested cut off value of 4% to diagnose beta-thalassemia trait, our study has used lower cutoff (Hb A_2 >3.5%). The cutoff value of hemoglobin A_2 (HbA_2) to diagnose beta-thalassemia trait can vary slightly depending on the laboratory and the population being studied. However, in general, a level of HbA_2 greater than 3.5% is often considered suggestive of beta-thalassemia trait. This is typically confirmed

through additional testing, such as hemoglobin electrophoresis or genetic testing, to definitively diagnose beta-thalassemia trait. It's important to note that interpretation of HbA_2 levels should be done in conjunction with clinical findings and other laboratory tests for a comprehensive diagnosis.[24–27]

Subjects with HbA_2 level at > 13% could be diagnosed as Hb E trait and also possible alpha-thalassemia disease, something that we should be aware of. Since we did not examine for alpha-thalassemia deletion and/or mutation, this cannot be well concluded. To distinguish between Hemoglobin E (HbE) trait and alpha-thalassemia trait, one must comprehend the genetic and hematological distinctions that exist between these two disorders. Both are types of thalassemia, hereditary blood illnesses characterised by decreased hemoglobin production, resulting in anemia. Colaco[26] discuss the genetic and diagnostic implications of borderline HbA_2 levels, which can be caused by various molecular defects. Nevertheless, they vary in terms of the particular globin chains that are impacted and their geographical distribution. In order to definitively distinguish between HbE trait and alpha thalassemia trait, medical professionals frequently utilise a combination of haematological tests, including a complete blood count (CBC), hemoglobin electrophoresis, and occasionally DNA analysis to identify genetic abnormalities or deletions. Individuals who are suspected of having these disorders should seek consultation with a healthcare professional or a hematologist in order to obtain an accurate diagnosis and appropriate management.

The prevalence of anemia among medical students varies depending on numerous factors, but being knowledgeable in medical science and having a sufficient socio-economic background might be caused by other than IDA. A study of medical students from other parts of Indonesia has shown that only 7.9% are anemic, similar to other studies in Malaysia. The low prevalence of anemia is also found in Malaysia among Malay (6.6%), Chinese (6.9%), and

Indian (26%).[17] Moreover, among medical students, anemia was observed in 33.4% of Pakistani students[28], in 28.3% of Indian students from Bangalore[29], and 52.7% in Nepal.[30]

Some studies mainly use CBCs from venous blood, while others use finger-pricking methods. These methods can produce different outcomes due to differences in hemoglobin measurement.[31–33] Venous blood is preferred for precise and accurate measurements of hemoglobin levels and other blood parameters. Fingerstick tests are used for point-of-care testing, rapid screenings, and immediate findings. However, they may need to be more precise in detecting blood disorders or making life-saving medical decisions. Medical experts often use venous blood testing for verification and more precise evaluation when clinical concerns arise.

Medical students, like many other young adults, can be susceptible to anemia due to dietary habits, lifestyle choices, and stress. The high cost of medical education at least indicates that their socio-economic status is quite good, however, they often have demanding schedules and face high levels of stress, which can contribute to poor eating habits, irregular meals, and inadequate nutrition. Moreover, prolonged studying periods and lack of physical activity can impact their health. Promoting healthy lifestyles, providing nutritious food options, encouraging physical activity, and raising awareness about maintaining good health can help mitigate the risk of anemia among medical students.[34,35]

Anemia affects more women than men, especially those of reproductive age, and is more prevalent in areas with high iron deficiency or parasite diseases. Female adolescents have lower hemoglobin levels than male adolescents, with anemia prevalence in women being 33.7% compared to 11.3% in men during reproductive years.[1,36] It was largely due to factors like menstrual blood loss in females and hormonal differences between the genders. Menstrual blood loss indeed contributes to lower hemoglobin levels in females compared to males. Testosterone, higher in males, doesn't directly impact hemoglobin levels but may reflect

differences in overall health. However, it's essential to note that lower hemoglobin levels in females aren't always indicative of underlying health issues; they can be a natural consequence of menstruation. Kannan[37] reported a high prevalence of anemia among female medical students, with a correlation to menstrual abnormalities and nutritional habits. Alsheikh[38] found a high prevalence of iron-deficiency anemia among Saudi female medical students, but no significant correlation with background, gynecological history, or dietary habits.

Male adolescents have higher testosterone levels, which typically rise during puberty, contributing to physical changes like muscle growth, deepening of the voice, and the development of male sexual characteristics. While testosterone itself doesn't directly cause anemia, certain conditions associated with hormonal imbalances or other health issues might impact hemoglobin levels in males. Anemia in male adolescents can occur due to various reasons unrelated to testosterone levels. Iron deficiency, chronic diseases, certain genetic conditions affecting red blood cell production, or nutritional deficiencies can lead to anemia in males.[39,40]

Thalassemia is a genetic blood disorder that affects hemoglobin synthesis. Thalassemia trait refers to thalassemia carriers with one defective and one normal hemoglobin gene. This defect results in reduced production of normal hemoglobin, leading to smaller and paler red blood cells (low MCV/MCH) but with normal overall hemoglobin levels (normal Hb). Microcytic anemia can occur if the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are both low. Iron deficiency, which can result from insufficient food intake, malabsorption, chronic or persistent blood loss, or increased demand for iron during pregnancy, is the most prevalent type of microcytic anemia. Other causes of microcytic anemia include hemoglobinopathy and sideroblastic anemia, a rare condition where the bone marrow produces abnormal red blood cells.[41–44]

Difficulty to distinguish IDA and alpha-thalassemia trait

Iron deficiency anemia (IDA) and alpha-thalassemia trait (TT) are common conditions of microcytic hypochromic anemia (MHA). Distinguishing IDA and the alpha-thalassemia trait can be challenging due to their similar symptoms and laboratory findings. Iron therapy, usually oral or intravenous iron supplementation, is the primary treatment for IDA. Monitoring the patient's response to iron therapy is crucial.[45] Close monitoring of the patient's response to iron therapy is crucial during the diagnostic trial. Self-administering iron supplements without proper diagnosis and supervision is not advisable, as excessive iron intake can have adverse health effects.[46] Alpha-thalassemia is a genetic condition affecting alpha-globin chain production in hemoglobin. Alpha-thalassemia trait carriers typically have mild or no symptoms, and their hemoglobin levels are usually within the normal range. The alpha-thalassemia trait does not require iron therapy, as it is unrelated to an iron deficiency. To differentiate between IDA and the alpha-thalassemia trait, healthcare providers often use various diagnostic tools, including blood tests, hemoglobin electrophoresis, and DNA analysis.[47] Hemoglobin electrophoresis can help identify the specific type of hemoglobin and reveal the presence of alpha-thalassemia. DNA analysis, such as polymerase chain reaction (PCR) or genetic testing, can definitively diagnose of alpha-thalassemia by detecting specific genetic mutations associated with this condition. If there is uncertainty in the diagnosis or a need for confirmation, further DNA examination can be valuable in determining the presence of alpha-thalassemia or other hemoglobinopathies. Both IDA and the alpha-thalassemia trait can coexist, making a precise diagnosis even more important.

Limitations of the study

The study's limitations include limited availability of examination kits for subjects with anemia and low MCV/MCH, and no iron therapy was given to those suspected of IDA. Iron therapy can be used as a diagnostic tool to help distinguish between IDA and the alpha-

thalassemia trait, especially when there is uncertainty in the diagnosis. The other limitation of this study was the small number of respondents, with a female majority. There are parameters related to RBC that are lower in females compared to males. We also did not ask in detail about the pattern of menstrual bleeding, whether it is heavy bleeding, therefore, we could not analyse the relation between menstrual bleeding and low ferritin.

CONCLUSION

Thalassemia is a genetic blood disorder characterized by abnormal hemoglobin production that results in reduced production of hemoglobin and red blood cells, leading to anemia. Low MCV and/or MCH (stage 1) are common indicators observed in thalassemia, but hemoglobin electrophoresis (stage 2) and DNA genotyping (stage 3) are two essential tests needed in diagnosing thalassemia. Genetic testing can identify specific mutations or changes in the genes responsible for hemoglobin production to confirm the presence of thalassemia and determine its type (alpha thalassemia or beta thalassemia) and severity. DNA analysis can also distinguish between iron deficiency anemia and the alpha-thalassemia trait, which has many similarities. In places where thalassemia is common, combining Hb analysis and DNA testing as the second and third stage tests is the best technique to determine a person's career status. However, a healthcare professional must make the diagnosis and treatment plan, as proper management depends on accurately identifying the underlying condition.

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Authors' contributions:

RW contributed in the study concept, secured grants for study funding, designing the study, recruitment of respondents, data analysis, and drafting the manuscript. IMN designing and conducting lab work for genetic analysis. EXT and AK managed the data collection and performed the statistical analysis. All authors took part in compiling references and contributed to constructing the research plan and giving insight into research implications for the near future study and policy, and finalizing the draft manuscript.

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The author declares that there are no significant competing financial, professional, or personal interests that might have affected the performance or presentation of the work described in this article.

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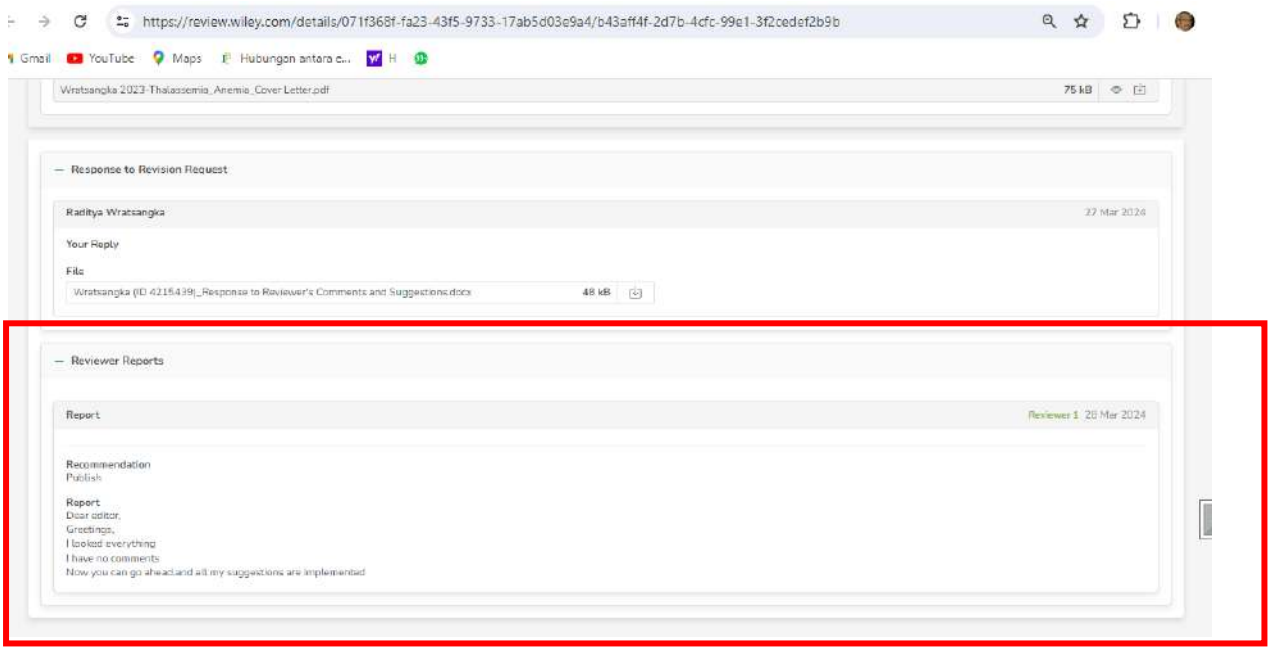
SPSS Data of this study are available at:
https://drive.google.com/drive/folders/1gjg7998MjKi9H2NVr-HoG4EgurO8FZC2?usp=drive_link

Respons Reviewer terhadap Resubmit Revised Article pertama (28 Maret 2024)



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4215439 - Revision Needed In The Manuscript

Saranya Ravi <saranya.ravi@hindawi.com> Sat, Mar 30, 2024 at 4:22 PM
Reply-To: Saranya Ravi <saranya.ravi@hindawi.com>
To: raditya@trisakti.ac.id

Dear Dr. Raditya Wratsangka,

This is regarding manuscript **4215439** titled "**Anemia among medical students from Jakarta - Indonesia: Iron deficiency or carrier thalassemia?**" submitted to the "**Anemia**".

While checking the manuscript at the QC stage, it was found that some of the references contain "***et al***" instead of a complete author list.

Kindly remove the "et al" from the references and provide the complete author list for all the references.

After making the required changes send the manuscript as an email attachment to me.

Best regards,
Saranya

_____

Saranya Ravi
Quality Checker

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4215439 - Revision Needed In The Manuscript

Dr. dr. Raditya Wratsangka, Sp.OG(K) <raditya@trisakti.ac.id> Sun, Mar 31, 2024 at 5:15 AM
To: Saranya Ravi <saranya.ravi@hindawi.com>

Dear Ms. Saranya Ravi,

Thank you for your checking and suggestion regarding our manuscript (**ID 4215439)** titled "**Anemia among medical students from Jakarta - Indonesia: Iron deficiency or carrier thalassemia?**" submitted to the "**Anemia**"

We have removed the "et al" from the references and provide the complete author list for all the references. (Ref no. 8, 17, 24, 25 and 46)
The manuscript we've revised is attached to this email.

Best regards,
Raditya Wratsangka
[Quoted text hidden]
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Dr. dr. Raditya Wratsangka, Sp.O.G, Subsp. Obginsos
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Anemia among medical students from Jakarta - Indonesia:

Iron deficiency or carrier thalassemia?

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ABSTRACT

BACKGROUND: Anemia, a global health concern, affects one-fourth of the global population, particularly women. In Indonesia, its prevalent is at 23.7%, with 32.0% among 15–24 year-olds. Factors include poor nutrition, infectious diseases, chronic diseases, inherited disorders, and inadequate healthcare access. This study aimed to investigate anemia prevalence and its etiology among medical students from Jakarta.
METHODS: This study was a descriptive research with a cross-sectional approach. Undergraduate students aged 18-23 years old were selected and consented to participate by a consecutive non-random sampling methods. Laboratory blood data were evaluated (including Hb, MCV, MCH, HbA_2, and Ferritin levels) and DNA was isolated to confirm the type of thalassemia carrier.
RESULTS: In total, 140 medical students, mainly female, were recruited. Anemia was found in 13.6% (11.4% had low MCV and/or MCH), and 16.5% had low MCV and/or MCH without anemia. Hb electrophoresis revealed high HbA_2 values, suggesting the HbE variant (2.1%), and β-thalassemia carrier (0.7%). DNA analysis confirmed *the cd26 mutation* and *heterozygous IVS1nt5*. Among those without anemia, 5% had *α-deletion*, while in the group with anemia, 1.4% had *α-deletion* (with coexistent IDA), 3.6% had *α-deletion*, and 0.7% had *β-mutation*.
CONCLUSION: DNA analysis can identify specific mutations associated with alpha-thalassemia, distinguishing between iron deficiency anemia and the alpha-thalassemia trait. Thalassemia screening should involve low MCV and/or MCH values as the first step (stage 1), followed by Hb analysis (stage 2) and DNA analysis (stage 3). In common areas, a combination of Hb and DNA testing is best. However, healthcare professionals must diagnose and treat thalassemia, as proper management relies on accurately identifying the underlying condition.

INTRODUCTION

Anemia is a worldwide public health issue, affecting both developed and developing nations. Anemia is also the third leading cause of years with lived disability (YLDs).[1] One-fourth of the global population is estimated to be anemic, with cases increasing rapidly in women. Over half a billion women between the ages of 15 and 49 make up the global anemia prevalence in 2019, including women of reproductive age (WRA; 29.9%), pregnant women (36.5%) and non-pregnant WRA (29.6%), with sub-Saharan Africa and South Asia having the most significant rates.[1] In 2021, there is an increase of 420 million cases over three decades.[1,2] Data in Indonesia revealed that the prevalence of anemia in Indonesia was 23.7%.[3] Additionally, the prevalence of anemia among individuals aged 15-24 years was 32.0%.

The high prevalence of anemia among young adults in Indonesia may differ among regions and population subgroups and is likely due to a combination of factors, including poor nutrition, infectious diseases, chronic diseases, inherited disorders (sickle cell anemia, thalassemia), and inadequate access to healthcare.[4,5] Interestingly, recent evidence has shown that the awareness programs and laboratory examinations have identified more thalassemia carriers, minors, or traits among young adults.[6]

Thalassemia carrier has a small red blood size (microcytic) as shown in low MCV values and/or less red blood cell color (hypochromic) as shown in low MCH values. Thalassemia carrier does not necessarily have low Hb. Anemia and thalassemia can cause low levels of hemoglobin in the blood, leading to low levels of red blood cells and decreased oxygen delivery to tissues.[7] Thalassemia is one condition that can cause anemia, however, thalassemia minor can be found without anemia.[7] The two conditions share some similarities, but have some essential differences.

Thalassemia is an inherited autosomal recessive gene disorder caused by a decreased or absent production of one or two types of globin chains, and classified according to the particular globin chain affected, such as α-globin and β-globin. Thalassemia is also a significant health issue in Indonesia, particularly among young adults.[8,9] Indonesia is located along the 'Thalassemia Belt', a geographic region that stretches from the Mediterranean to Southeast Asia, where the prevalence of thalassemia is highest.[10–12]

Medical students, as part of the young adult population, are obliged to study various diseases, including anemia and thalassemia in their courses on genetics, hematology, and other related topics. Medical students' knowledge, attitudes, and practice towards thalassemia are likely to be shaped by various factors, including their academic and personal backgrounds, their exposure to patients with thalassemia, and their understanding of the social and cultural contexts in which the disease occurs. Medical students who look physically fit may not even know their status of anemia and carriers of thalassemia traits.[13–16] Study in Malaysia involving 400 undergraduate medical students has reported that 14.5% students had hypochromic microcytic red cell indices, 11% showed thalassemia red cell indices, and only 3.5% had iron deficiency (IDA) red cell index which were finally confirmed by serum iron/TIBC analysis.[17]

Therefore, it is interesting to also explore the etiology of anemia among students in Indonesia compared to the neighboring country Malaysia. The study aimed to explore the anemia prevalence and etiology among medical students from Jakarta.

MATERIAL AND METHOD

Study design

This study was descriptive research with a cross-sectional approach to explore the anemia prevalence among medical students and the etiology of the anemia. Laboratory blood data were evaluated (including Hb, MCV, MCH, HbA_2, and Ferritin levels) and DNA was isolated to

confirm the type of thalassemia carrier (trait). The study was approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Trisakti (No. 011/KER/FK/IV/2021).

Subjects

Undergraduate students of the Faculty of Medicine, Universitas Trisakti, aged 18-23 were selected and consented to participate by a consecutive non-random sampling methods. Those who had a history of chronic disease and had received blood transfusions in the past three months were excluded from this study.

Complete blood count and HbA_2 measurement

Venous blood sampling was collected in a 3 ml EDTA tube to be checked further for a complete blood count (CBC), followed by Ferritin measurement and HbA_2 determination by High Performance Liquid Chromatography (Bio-Rad). CBC was analyzed using the Flow Cytometry Method Using a Semiconductor Laser (Sysmex-XN®); ferritin measurement was analyzed using the chemiluminescent magnetic microparticle immunoassay (CMIA) method (*Architect® i2000*). The definition of anemia is according to WHO standards based on sex (men <13g/dL and women <12g/dL). The red blood cell (RBC) indices were then analyzed based on a guide from the Ministry of Health, Republic of Indonesia, MCV< 80 fL and MCH < 27pg values which were used as initial thalassemia screening. Furthermore, Hb analysis data were classified based on HbA_2 fraction values according to the protocol of the manufacturer (Bio-Rad) where < 2.0% suggested for α-thalassemia carriers; 2.0–3.5% as normal value; >3.5% was indicated as β-thalassemia carriers; and very high HbA_2 level peak shown indicated HbE carriers. Hereafter, subjects who had normal HbA_2 but had low/normal Hb and/or low MCV and/or low MCH were examined further for DNA analysis. Those with low MCV and/or MCH and with normal HbA2 value (2-3.5%) were further confirmed with DNA examination to distinguish the etiology of anemia whether due to iron deficiency or alpha thalassemia carrier. The students

were also checked for their ferritin levels to determine whether anemia was due to iron deficiency

DNA genotyping identification

Subjects with low MCV and/or MCH, low and normal Hb, and low and normal ferritin were identified by the level of HbA2 and subsequently confirmed their genotype by DNA analysis (N = 39). Due to the limited availability of examination kits, DNA tests were carried out on 30 of 39 subjects, whose selection was done by simple random sampling.

We used the α-Globin StripAssay® (Ref. 4-160, ViennaLab Diagnostics GmbH) to detect 21 common α-globin mutations in separated teststrips. The procedure includes three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers, (3) hybridization of amplification products to teststrips containing allele-specific oligonucleotide probes immobilized as an array of parallel line. The bound biotinylated sequences on specific wild types and mutants are detected using streptavidin-alkaline phosphatase and color substrates. We prepared three separate PCR mixtures (A1, A2, and B), following both PCR conditions and hybridization according to the manufacturer's instructions. We analyzed the hybridization results by comparing the line with the provided CollectorTM sheet. In heterozygous conditions, both mutated bands and normal bands will appear. In homozygous mutations, only the positive band is colored. If no mutations are contained in the StripAssay panel, only PCR and normal controls are colored. Furthermore, to identify β-globin gene mutations, we used the β-Globin StripAssay® SEA (Ref. 4-150, Vienna Lab Diagnostics GmbH). The assay covers 22 common β-globin mutations in one strip only. Unlike the assay for α-thalassemia, the assay for β-thalassemia was only in one PCR mixture and followed the manufacturer's instructions. The hybridization procedure and analysis of test strips results were the same as in α-globin gene mutation detection.

RESULT

In total, 140 medical students were recruited, primarily female (69.3%; n=97), with a median age of 20 (range 18–26 years). The level of Hb in this study is 12.7 g/dL among female and 15.7 g/dL among male students. Anemia was found in 13.6% (n=19) of the female students (table 1). Interestingly, 16.5% of students (n=23; 18 females and 5 males) were not anemic but had low MCV and/or low MCH. These students were subjected to the next level of the thalassemia screening program as indicated by the Ministry of Health. In addition, 16 (11.4%) students who were anemic and had low MCV and/or MCH were examined further to determine the etiology of anemia.

Table (1). Hemoglobin level based on gender and MCV and/or MCH level in medical students (n=140)

	Level of Hemoglobin		
	Not Anemia n (%)	Anemia n (%)	Total n (%)
Male	43 (30.7)	0	43 (30.7)
MCV and/or MCH			
• Normal	38 (27.1)	0	38 (27.1)
• Low	5 (3.6)*	0	5 (3.6)
Female	78 (55.7)	19 (13.6)	97 (69.3)
MCV and/or MCH			
• Normal	60 (42.9)	3 (2.1)	63 (45.0)
• Low	18 (12.9)*	16 (11.4)**	34 (24.3)

Note: Anemia Hb: <13.0 g/dL (male); < 12.0 g/dL (female)
Low MCV: <80 fL
Low MCH: <27 pg

* Not anemia but with low MCV and/or MCH, subjected for further examination

** Anemia and were further examined to explore the etiology of anemia

In our study, the students were examined by Hb electrophoresis (level 2), resulting in a very high HbA2 value (>13%), suggesting an HbE variant (2,1%; n=3); and a high HbA2 value (>4% to 13%), indicating β-thalassemia carrier (0.7%; n=1); that was confirmed by DNA analysis (level 3), resulting in cd26 mutation and heterozygous IVS1nt5, respectively (table 2).

Table (2). Complete blood count examination (Level 1) followed by Hb variant A2 (Level 2) and DNA confirmation (Level 3) among medical students from a private university in Jakarta (n=140)

Level 1		Level 2		
		HbA2		
MCV and/or MCH	Hb level	2 – 3.5% n (%)	> 3.5% n (%)	>>> 13% n (%)
Low (n=39)	Not Anemia	20 (14.3) ***	0	3 (2.1) *
	Anemia	15 (10.7) ****	1 (0.7) **	0
Normal (n=101)	Not Anemia	98 (70.0)	0	0
	Anemia	3 (2.1)	0	0

Note:
*Cd26 (G>A) heterozygote;
**IVS1nt5 heterozygote;
***Further DNA analysis needed whether iron deficiency anemia or alpha Thalassemia carrier
****Need iron therapy first

Among those without anemia (n=15), 5% (n=7) had α-deletion (table 3). Furthermore, in a group with anemia (n=14), α-deletion (with coexistent IDA) was detected in 1.4% (n=2), αdeletion 3.6% (n=5), and β-mutation in 0.7% (n=1).

Table (3). Evaluation of Ferritin among students (n=30) with low MCV and/or MCH (Level 1) in relation to Hemoglobin level, HbA2 (Level 2) and DNA genotype (Level 3)

Level 1 (Low MCV/MCH) N = 30	Level 2 Hb Electrophoresis ($Hb A_2$) 2-3.5	> 3.5	>>>	Level 3 DNA Genotype
• **Not Anemia** (n = 16)				
• Ferritin Low (n = 1)	1			Beta (β): IVS1-nt5 [G>C] heterozygote + coexistent IDA ***)
			3	Confirmed HbE: codon 26 [G>A] heterozygote
• Ferritin Normal (n = 15) *)	4			No alpha (α) deletion or mutation found, need further exploration
	4			Alpha (α): Single α globin gene deletion 3.7 kb type heterozygote
	3			Alpha (α): Double α globin gene deletion SEA type heterozygote
• **Anemia** (n = 14)				
	4			No-α deletion or mutation; suggesting IDA
• Ferritin Low (n = 6)	1			Alpha (α): 3.7 kb triplicated α gene heterozygote + coexistent IDA ***)
	1			Alpha (α): α2 cd 59 [GGC>GAC] heterozygote + coexistent IDA ***)
	3			Alpha (α): Double α globin gene deletion SEA type heterozygote
• Ferritin Normal (n = 8) **)	1			Alpha (α): α2 cd 142 [TAA>CAA] (Hb Constant Spring) heterozygote
	1			Alpha (α): α2 cd 59 [GGC>GAC] heterozygote
		1		Beta (β): IVS1-nt5 [G>C] heterozygote

Note. Anemia was designated as Hb < 12.0 g/dL for females and Hb < 13.0 g/dL for males
*) One sample DNA examination failed
**) Two samples DNA examination failed
***) Point of interest; IDA with coexistence confirmed Alpha (ά)

DISCUSSION

Our study has shown that among medical students aged 18 to 23 years old, anemia has been detected in 13.6% (n=19) students, all females. Interestingly, 16.5% of students (18 females and 5 males) who are not anemic, have low MCV and/or MCH. All these students have been examined for ferritin, Hb electrophoresis, and DNA genotyping for alpha and beta-globin to explore the etiology of anemia.

If a female is experiencing mild anemia but is otherwise in good physical condition, it's essential to consider the potential causes and appropriate management strategies for her condition. Anemia, characterized by a decrease in the number of red blood cells or the amount of hemoglobin in the blood, can lead to various symptoms, including fatigue, weakness, pale

skin, and shortness of breath, among others. However, mild cases may have minimal or no symptoms, especially if the individual is generally healthy and physically active.[18]

Medical students who have a middle-class socio-economic status and are knowledgeable about anemia and thalassemia should be aware of the effects of poor nutrition and inadequate dietary iron intake. Not consuming enough iron-rich foods (including red meat, poultry, fish, beans, lentils, tofu, fortified cereals, and green leafy vegetables) in their diet can lead to IDA, which is assumed to be low.[19–21] However, the results showed that 13.6% (n=19) had anemia and additionally, 16.5% (n=23) were not anemic but had low MCV/MCH.

Anemia characterized by a normal MCV/MCH is known as normocytic or normochromic anemia. This form of anemia is distinguished by red blood cells that are of average size (normocytic) and contain a normal amount of hemoglobin (normochromic). The causes can vary and encompass: acute blood lost, chronic diseases, aplastic anemia, hemolytic anemias, anemia of persistent disease, renal anemia, bone marrow disorders, and endocrine problems such as hypothyroidism or hypopituitarism. Diagnosis of the specific cause of normocytic, normochromic anemia involves a thorough medical history, physical examination, and a series of laboratory tests beyond just the complete blood count (CBC) that measures MCV and MCH, such as reticulocyte count, serum iron levels, ferritin, total iron-binding capacity (TIBC), vitamin B12, and folate levels, as well as tests for renal function and possible bone marrow examination.

Patients with low MCV/MCH values typically exhibit signs of microcytic and hypochromic anemias, which are often associated with iron deficiency or disorders of hemoglobin synthesis like thalassemia. However, if ferritin levels are normal and there are no mutations in the DNA for alpha- and beta-globin genes (which would indicate thalassemia), other potential causes could include: anemia of chronic disease, lead poisoning, sideroblastic anemia, copper deficiency, rare hemoglobin variants, and chronic kidney disease. It's important

to note that the diagnosis of anemia and its underlying cause is complex and requires a comprehensive approach, including detailed patient history, physical examination, and a broader panel of laboratory tests beyond MCV, MCH, and ferritin levels. Consulting with a hematologist may be beneficial for patients with unexplained anemia.

Indonesia, located in the thalassemia belt area, has many thalassemia careers in Southeast Asia, making anemia in young adulthood a significant concern. However, no result of non-severe thalassemia disease has been found among medical students in this study. Anemia in young adults can have other causes, such as hemoglobin abnormalities or hemoglobinopathy, as the most common causes. Efforts to detect early thalassemia careers have not been carried out systematically. Around 3.0-10.0% of the Indonesian population carry β-thalassemia (β-thal) and 2.6-11.0% of the population carry α-thalassemia (α-thal), with the highest incidence rates reported in regions such as Bali, East Nusa Tenggara, and Maluku.[22] It is estimated that around 2500 babies are born with β-thal major (β-TM) each year.[22].

Iron Deficiency Anemia or Thalassemia Carrier?

Further analysis with Hb electrophoresis has shown that it is difficult to distinguish between IDA and alpha-thalassemia carriers. Both conditions can lead to microcytic (small red blood cells) and hypochromic (pale) red blood cells, making it necessary to perform additional tests, such as DNA examination, to make a definitive diagnosis. In restricted facilities, MCV<80 fl and/or MCH<27 pg is the optimal first-round mass screening approach for β-thalassemia carriers. Gradually implement Hb electrophoresis, and DNA analysis should be done in many regions to confirm mutations for accurate carrier detection wherever possible.[23] Since the machine BioRad Variant that we used has suggested cut off value of 4% to diagnose beta-thalassemia trait, our study has used lower cutoff (Hb A_2 >3.5%). The cutoff value of hemoglobin A_2 (HbA_2) to diagnose beta-thalassemia trait can vary slightly depending on the

laboratory and the population being studied. However, in general, a level of HbA_2 greater than 3.5% is often considered suggestive of beta-thalassemia trait. This is typically confirmed through additional testing, such as hemoglobin electrophoresis or genetic testing, to definitively diagnose beta-thalassemia trait. It's important to note that interpretation of HbA_2 levels should be done in conjunction with clinical findings and other laboratory tests for a comprehensive diagnosis.[24–27]

Subjects with HbA_2 level at > 13% could be diagnosed as Hb E trait and also possible alpha-thalassemia disease, something that we should be aware of. Since we did not examine for alpha-thalassemia deletion and/or mutation, this cannot be well concluded. To distinguish between Hemoglobin E (HbE) trait and alpha-thalassemia trait, one must comprehend the genetic and hematological distinctions that exist between these two disorders. Both are types of thalassemia, hereditary blood illnesses characterised by decreased hemoglobin production, resulting in anemia. Colaco[26] discuss the genetic and diagnostic implications of borderline HbA_2 levels, which can be caused by various molecular defects. Nevertheless, they vary in terms of the particular globin chains that are impacted and their geographical distribution. In order to definitively distinguish between HbE trait and alpha thalassemia trait, medical professionals frequently utilise a combination of haematological tests, including a complete blood count (CBC), hemoglobin electrophoresis, and occasionally DNA analysis to identify genetic abnormalities or deletions. Individuals who are suspected of having these disorders should seek consultation with a healthcare professional or a hematologist in order to obtain an accurate diagnosis and appropriate management.

The prevalence of anemia among medical students varies depending on numerous factors, but being knowledgeable in medical science and having a sufficient socio-economic background might be caused by other than IDA. A study of medical students from other parts of Indonesia has shown that only 7.9% are anemic, similar to other studies in Malaysia. The

low prevalence of anemia is also found in Malaysia among Malay (6.6%), Chinese (6.9%), and Indian (26%).[17] Moreover, among medical students, anemia was observed in 33.4% of Pakistani students[28], in 28.3% of Indian students from Bangalore[29], and 52.7% in Nepal.[30]

Some studies mainly use CBCs from venous blood, while others use finger-pricking methods. These methods can produce different outcomes due to differences in hemoglobin measurement.[31–33] Venous blood is preferred for precise and accurate measurements of hemoglobin levels and other blood parameters. Fingerstick tests are used for point-of-care testing, rapid screenings, and immediate findings. However, they may need to be more precise in detecting blood disorders or making life-saving medical decisions. Medical experts often use venous blood testing for verification and more precise evaluation when clinical concerns arise.

Medical students, like many other young adults, can be susceptible to anemia due to dietary habits, lifestyle choices, and stress. The high cost of medical education at least indicates that their socio-economic status is quite good, however, they often have demanding schedules and face high levels of stress, which can contribute to poor eating habits, irregular meals, and inadequate nutrition. Moreover, prolonged studying periods and lack of physical activity can impact their health. Promoting healthy lifestyles, providing nutritious food options, encouraging physical activity, and raising awareness about maintaining good health can help mitigate the risk of anemia among medical students.[34,35]

Anemia affects more women than men, especially those of reproductive age, and is more prevalent in areas with high iron deficiency or parasite diseases. Female adolescents have lower hemoglobin levels than male adolescents, with anemia prevalence in women being 33.7% compared to 11.3% in men during reproductive years.[1,36] It was largely due to factors like menstrual blood loss in females and hormonal differences between the genders. Menstrual blood loss indeed contributes to lower hemoglobin levels in females compared to males.

Testosterone, higher in males, doesn't directly impact hemoglobin levels but may reflect differences in overall health. However, it's essential to note that lower hemoglobin levels in females aren't always indicative of underlying health issues; they can be a natural consequence of menstruation. Kannan[37] reported a high prevalence of anemia among female medical students, with a correlation to menstrual abnormalities and nutritional habits. Alsheikh[38] found a high prevalence of iron-deficiency anemia among Saudi female medical students, but no significant correlation with background, gynecological history, or dietary habits.

Male adolescents have higher testosterone levels, which typically rise during puberty, contributing to physical changes like muscle growth, deepening of the voice, and the development of male sexual characteristics. While testosterone itself doesn't directly cause anemia, certain conditions associated with hormonal imbalances or other health issues might impact hemoglobin levels in males. Anemia in male adolescents can occur due to various reasons unrelated to testosterone levels. Iron deficiency, chronic diseases, certain genetic conditions affecting red blood cell production, or nutritional deficiencies can lead to anemia in males.[39,40]

Thalassemia is a genetic blood disorder that affects hemoglobin synthesis. Thalassemia trait refers to thalassemia carriers with one defective and one normal hemoglobin gene. This defect results in reduced production of normal hemoglobin, leading to smaller and paler red blood cells (low MCV/MCH) but with normal overall hemoglobin levels (normal Hb). Microcytic anemia can occur if the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are both low. Iron deficiency, which can result from insufficient food intake, malabsorption, chronic or persistent blood loss, or increased demand for iron during pregnancy, is the most prevalent type of microcytic anemia. Other causes of microcytic anemia include hemoglobinopathy and sideroblastic anemia, a rare condition where the bone marrow produces abnormal red blood cells.[41–44]

Difficulty to distinguish IDA and alpha-thalassemia trait

Iron deficiency anemia (IDA) and alpha-thalassemia trait (TT) are common conditions of microcytic hypochromic anemia (MHA). Distinguishing IDA and the alpha-thalassemia trait can be challenging due to their similar symptoms and laboratory findings. Iron therapy, usually oral or intravenous iron supplementation, is the primary treatment for IDA. Monitoring the patient's response to iron therapy is crucial.[45] Close monitoring of the patient's response to iron therapy is crucial during the diagnostic trial. Self-administering iron supplements without proper diagnosis and supervision is not advisable, as excessive iron intake can have adverse health effects.[46] Alpha-thalassemia is a genetic condition affecting alpha-globin chain production in hemoglobin. Alpha-thalassemia trait carriers typically have mild or no symptoms, and their hemoglobin levels are usually within the normal range. The alpha-thalassemia trait does not require iron therapy, as it is unrelated to an iron deficiency. To differentiate between IDA and the alpha-thalassemia trait, healthcare providers often use various diagnostic tools, including blood tests, hemoglobin electrophoresis, and DNA analysis.[47] Hemoglobin electrophoresis can help identify the specific type of hemoglobin and reveal the presence of alpha-thalassemia. DNA analysis, such as polymerase chain reaction (PCR) or genetic testing, can definitively diagnose of alpha-thalassemia by detecting specific genetic mutations associated with this condition. If there is uncertainty in the diagnosis or a need for confirmation, further DNA examination can be valuable in determining the presence of alpha-thalassemia or other hemoglobinopathies. Both IDA and the alpha-thalassemia trait can coexist, making a precise diagnosis even more important.

Limitations of the study

The study's limitations include limited availability of examination kits for subjects with anemia and low MCV/MCH, and no iron therapy was given to those suspected of IDA. Iron

therapy can be used as a diagnostic tool to help distinguish between IDA and the alpha-thalassemia trait, especially when there is uncertainty in the diagnosis. The other limitation of this study was the small number of respondents, with a female majority. There are parameters related to RBC that are lower in females compared to males. We also did not ask in detail about the pattern of menstrual bleeding, whether it is heavy bleeding, therefore, we could not analyse the relation between menstrual bleeding and low ferritin.

CONCLUSION

Thalassemia is a genetic blood disorder characterized by abnormal hemoglobin production that results in reduced production of hemoglobin and red blood cells, leading to anemia. Low MCV and/or MCH (stage 1) are common indicators observed in thalassemia, but hemoglobin electrophoresis (stage 2) and DNA genotyping (stage 3) are two essential tests needed in diagnosing thalassemia. Genetic testing can identify specific mutations or changes in the genes responsible for hemoglobin production to confirm the presence of thalassemia and determine its type (alpha thalassemia or beta thalassemia) and severity. DNA analysis can also distinguish between iron deficiency anemia and the alpha-thalassemia trait, which has many similarities. In places where thalassemia is common, combining Hb analysis and DNA testing as the second and third stage tests is the best technique to determine a person's career status. However, a healthcare professional must make the diagnosis and treatment plan, as proper management depends on accurately identifying the underlying condition.

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Authors' contributions:

RW contributed in the study concept, secured grants for study funding, designing the study, recruitment of respondents, data analysis, and drafting the manuscript. IMN designing and conducting lab work for genetic analysis. EXT and AK managed the data collection and performed the statistical analysis. All authors took part in compiling references and contributed to constructing the research plan and giving insight into research implications for the near future study and policy, and finalizing the draft manuscript.

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Declaration of conflicting interests:

The author declares that there are no significant competing financial, professional, or personal interests that might have affected the performance or presentation of the work described in this article.

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SPSS Data of this study are available at:
https://drive.google.com/drive/folders/1gjg7998MjKi9H2NVr-HoG4EgurO8FZC2?usp=drive_link

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Dr. dr. Raditya Wratsangka, Sp.OG-K <raditya@trisakti.ac.id>

4215439 - Revision Needed In The Manuscript

Saranya Ravi <saranya.ravi@hindawi.com> Mon, Apr 1, 2024 at 3:16 PM
Reply-To: Saranya Ravi <saranya.ravi@hindawi.com>
To: raditya@trisakti.ac.id

Dear Dr. Raditya Wratsangka,

Thanks for your mail. We will check the file and then get back to you.

Best regards,
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On Sat, 30 Mar at 10:19 PM , Dr. dr. Raditya Wratsangka, Sp. OG(K) <raditya@trisakti.ac.id> wrote:

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We have removed the "et al" from the references and provide the complete author list for all the references. (Ref no. 8, 17, 24, 25 and 46)
The manuscript we've revised is attached to this email.

Best regards,
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On Sat, Mar 30, 2024 at 4:22 PM Saranya Ravi <saranya.ravi@hindawi.com> wrote:

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4215439: Your manuscript has been accepted for publication

Anemia <anemia@hindawi.com> Mon, Apr 1, 2024 at 3:22 PM
Reply-To: Anemia <lmatel@wiley.com>
To: Raditya Wratsangka <raditya@trisakti.ac.id>

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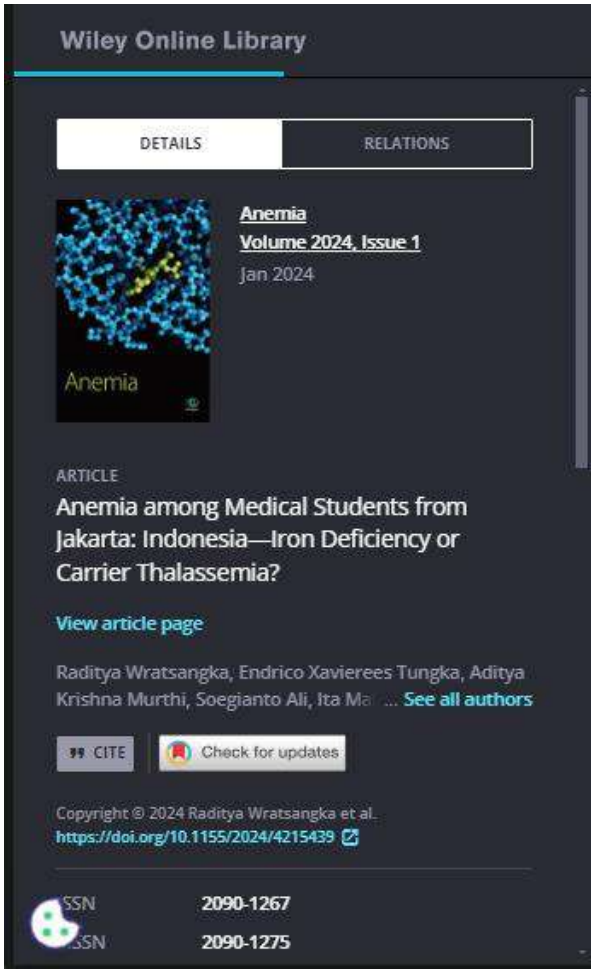

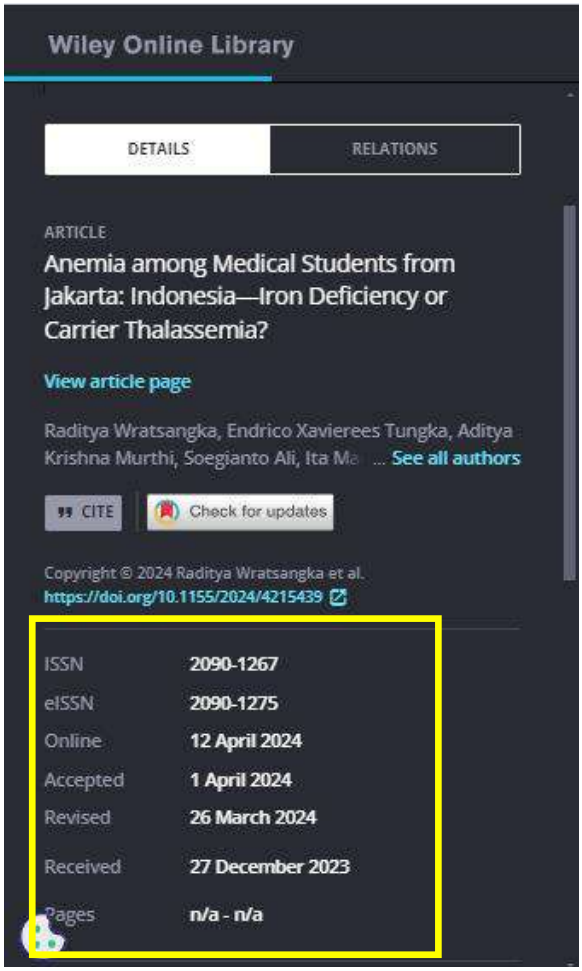

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Research Article

Anemia among Medical Students from Jakarta: Indonesia—Iron Deficiency or Carrier Thalassemia?

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