The pattern of iron deficiency with and without anemia among medical college girl students in high altitude southern Saudi Arabia

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ABSTRACT

Introduction: The prevalence of iron deficiency, latent and symptomatic, is heterogeneous worldwide. In this study, we aim to explore the prevalence of iron deficiency, with and without anemia, among medical college females at the high altitude Aseer region of Southwestern Saudi Arabia. Methodology: 200 female medical students were randomly sampled, between the ages of 19 and 27 years. Blood samples were collected for complete blood count (CBC) values and serum ferritin determination. Questionnaires were completed in order to collect demographics, medical history, and socioeconomic information of the participants. Results: Prevalence of overall iron deficiency was high (63%, serum ferritin <20 µg/L, 52.5%, ferritin <15 µg/L). Anemia, adjusted for high altitude (defined as less than 13 g/dL) was present in 41 participants (20.5%) and 12 (6.5%) have Hb <12 g/dL. Iron-deficiency anemia (Hb <13 g/dL and serum ferritin <15 µg/L) was present in 35 (17.5%). Personal and family history of anemia and poor animal product containing meals were positively correlated with the presence of iron deficiency state. Neither symptoms of anemia, nor the presence of menorrhagia correlate with the presence of iron deficiency anemia. Conclusion: Iron deficiency with and without anemia is a very common and condition in young females’ population at high altitude. Implementing a lab method to screen for anemia on vulnerable populations is needed. Frequently asymptomatic, the primary care providers should maintain a high degree of suspicion in order to initiate screening for iron status.

Keywords: Anemia, female, hemoglobin, high altitude, nutritional anemia, prevalence

Introduction

Iron deficiency is the commonest micronutrient deficiency reported globally. It is especially common among the female population of childbearing age. Iron deficiency with and without anemia is associated with a decrease in school and college attendance. In a recent study conducted in Saudi Arabia, the prevalence of iron deficiency among female medical students was found to be high. This study aimed to explore the prevalence of iron deficiency, with and without anemia, among medical college females at the high altitude Aseer region of Southwestern Saudi Arabia. The study sampled 200 female medical students between the ages of 19 and 27 years. Blood samples were collected for complete blood count (CBC) values and serum ferritin determination. Questionnaires were completed to collect demographics, medical history, and socioeconomic information.

Results: Overall, iron deficiency was prevalent with 63% of participants having a serum ferritin level <20 µg/L and 52.5% having a ferritin level <15 µg/L. Anemia, adjusted for high altitude (defined as a hemoglobin level <13 g/dL), was present in 41 participants (20.5%), with 12 (6.5%) having a hemoglobin level <12 g/dL. Iron-deficiency anemia (defined as a hemoglobin level <13 g/dL and serum ferritin level <15 µg/L) was present in 35 (17.5%) of the participants. Personal and family history of anemia and poor animal product-containing meals were positively correlated with the presence of iron deficiency. Neither symptoms of anemia nor the presence of menorrhagia correlated with the presence of iron deficiency anemia.

Conclusion: Iron deficiency with and without anemia is a very common condition in young females in the high altitude region of Aseer, Saudi Arabia. Implementing a lab method to screen for anemia on vulnerable populations is necessary. Since iron deficiency is often asymptomatic, primary care providers should maintain a high level of suspicion to initiate screening for iron status.

Keywords: Anemia, female, hemoglobin, high altitude, nutritional anemia, prevalence

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performances, both cognitively and physically. The prevalence of iron deficiency with and without anemia worldwide is heterogeneous. Iron deficiency prevalence of 23% among schoolgirls have been documented in some regions of Saudi Arabia.[10] However, there are no sufficient numbers of local research studies specifically focusing on and exploring the magnitude of the problem in the southwestern high-altitude regions like the Aseer region. It is quite possible that if our study demonstrated a higher than expected prevalence, we could inform the local health authorities to implement screening and treatment interventions.

Anemia is a major public health issue with the preponderance of 9% in developed nations and 43% in developing countries.[3] It is commonly observed in peoples at any stage of life, although women at childbearing age and young children are more susceptible, which may increase the risk of impaired cognitive and physical development and further increase mortality and morbidity rate.[3] Anemia has several causes such as nutritional (iron, folic acid, and vitamin B12), inherited (sickle cell and thalassemia), autoimmune (hemolytic anemia), infectious (malaria), demographic factors related (age and gender), malabsorption (achlorhydria), environmental pollutants (lead), socioeconomic (low maternal level of education and low household income), and chronic diseases (cancer).[8] Iron deficiency anemia (IDA) is the commonest among all types of anemia.[8] As reported by the World Health Organization (WHO), anemia among the population is defined as a hemoglobin concentration of (at sea level) <12 g/dL for nonpregnant women aged 15 years and above, and a hemoglobin concentration of < 11 g/dL for pregnant women.[8] The WHO suggested an adjustment factor when defining anemia for people residing at different high altitude levels.[9]

The prevalence of global anemia is estimated to be 496 million among nonpregnant women and 32.4 million among pregnant women aged 15–49 years.[7] Furthermore, twenty percent of maternal deaths are caused by anemia in half a billion women of reproductive age globally. IDA is still considered as the most prevalent and commonest type of micronutrient deficiency in the developing world,[8] which is reported as a result of long-term negative iron balance. Commonly, iron deficiency develops slowly and does not show any specific type of clinically apparent presentation until anemia becomes severe in nature.[9]

The etiology of iron deficiency anemia during adolescence might be considered due to the increased level of iron demand, blood loss and/or reduced iron intake, chronic blood loss, iron malabsorption (e.g., celiac disease), parasitic infection, or pregnancy, which may further lead to a considerable reduction in intellectual prowess and work and learning difficulties.[10] Poor social and physical activity, and mental and educational achievements among children that have a strong relationship with IDA may also continue into their adulthood and result in a low work efficiency, which may reflect in futuristic effects on economic productivity.[10] As far as the Middle East region is concerned, the reported prevalence of iron deficiency anemia in rural and urban people differs from 17% to 70% between pre-school children; 12.6% to 50% between school children; 14% to 42% between adolescents; and 11% to 54% between pregnant women.[11] Medical literature available on anemia focusing on adolescents and youth population is scanty, as compared to that focusing on women and child population. In Saudi Arabia, the incidence of iron deficiency anemia is 30%–56%.[12] A cross-sectional study performed among schoolgirls in Riyadh city demonstrated that the incidence of IDA was 40.5% among female adolescents aged 16–18 years old.[13]

The present study is an attempt to focus on prevalence and etiologies of iron deficiency and IDA in the female medical students at high altitude Aseer region in Southwestern Saudi Arabia. The clinical relations of iron deficiency are well-discussed with a vision to highlight the need for the establishment of a nationalized program to beat the nutritional deficiencies.

**Methodology**

**Study design**

This was a cross-sectional study intended to estimate the prevalence of iron store status (latent iron deficiency and iron deficiency anemia) among the female medical undergraduate students of all medical colleges by evaluating blood samples to obtain complete blood count (CBC) and serum ferritin (SF). Furthermore, a questionnaire was completed with students to further assess their overall health status, lifestyle, and socio-economic behavior in order to study any correlation with an iron deficiency if present. The study was conducted by the Department of Medicine, College of Medicine, King Khalid University and Affiliated hospital – Aseer Central Hospital between the time period of January 2019 and March 2019.

**Study participants**

The study targeted accessible main medical colleges complex, located in Abha city (the capital city of Aseer region located at 2270 m above sea level, with areas as high as 3000 m are also inhabited), for recruiting participants due to its proximity to the hospital and feasibility of taking and processing blood samples. The total number of students at the time of the study was 2614 students. A sample size of 200–250 participants was estimated based on that the prevalence of iron deficiency anemia is 11% and latent iron deficiency of 25% with a precision of 7% and 95% confidence level. The study was advertised as a study to explore nutrient deficiencies and associated anemia. The present study included a total of 200 female medical students voluntarily recruited between the ages of 19 and 27 years. The study protocol was approved by the Ethics Committee of Aseer Central Hospital ACH IRB No. 20181004. All students were well-informed in advance about the purpose of the study and protocol. Written informed consent was signed by all participants in advance.
Data collection
A voluntary Questionnaire was sent by email – after blood samples collection, to each female medical student to assess their dietary habits, socioeconomic status, demographic data, and if any current or previous medical history if present.

Laboratory evaluation
Venous blood was collected from every student and divided into 2 tubes; 2 mL was taken into K3EDTA tubes for the CBC test while 3 mL was drawn in a plain tube to determine serum ferritin (SF) level. Serum ferritin level had been chosen, as the single most sensitive indicator, to assess iron status in order to minimize the cost with the option to do further testing only if needed.

Hematological and biochemical measurement
Hemoglobin (Hb), reticulocytes count (RET), white blood cell count (WBC), red blood cell count (RBC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and platelets were calculated using the Sysmex xn-1000 hematology analyzer.

The serum ferritin was analyzed using the Unicell DXI 600 access immunoassay system.

Statistical analysis
Statistical analysis of the data was achieved with the help of the Statistical Package for Social Sciences (SPSS) version 21. All quantitative variables were examined for normality before analysis. Continuous variables were presented as mean and standard deviations. Independent sample t-test and one-way analysis of variance (ANOVA) were used to compare the mean and median proportions between IDA and nonanemic students for parameters like serum ferritin, hemoglobin (Hb), and mean cell volume (MCV). Differences between proportions were considered statistically significant if 95% CI did not overlap. "P" at or <0.05 is considered significant; "P" at or <0.01 and <0.001 are considered highly significant. Accordingly, "P" value >0.05 is considered nonsignificant In categorical variables, percentages and frequency counts were presented using a cross-tabulation test. Pearson's Chi-square test was used to investigate the association between the dependent variables (IDA) and the independent variables were grouped as socioeconomic information and dietary.

We defined latent iron deficiency (evidence of empty iron store but with no anemia) as serum ferritin (SF) levels less than 20 ng/L when anemia is absent. We define iron deficiency anemia as (reported Hb <13 g/dL) along with SF <15 ng/L.

Anemia in our participants (young, nonpregnant female) was defined as a Hb <13 g/dL. This is based on the WHO recommendation, to apply a correction factor when defining anemia at higher altitudes (adjustment factor of 1 g/dL for our high altitude area 2270 m above sea level). We also reported anemia without correction using a lower hemoglobin threshold of 12 g/dL. We used serum ferritin cut off of 15 ng/L as per the WHO recommendation.

Results
Table 1 summarizes the characteristics of the study participants. The age of the study participants ranged from 19 to 27 years. Most of the participants were from the highland Aseer region and all are longtime dwellers in Abha city.

Table 2 summarizes the finding of the study. Latent iron deficiency, defined as deficient iron stores (serum ferritin <15 ng/L) were found in 105 (52.5%), 35 (17.5%) of which have Hb <13 g/dL, giving an estimated prevalence of iron deficiency anemia of 17.5% [Figure 1].

Overall, Hb level <13 g/dL was found in 41 participants (35.5%), and Hb <12 g/dL was found in 13 participants (6.5%). Microcytosis, defined as MCV <80 fl was present in 125 (62.5%) participants. Roughly 50% of those with microcytosis has normal iron store (35.5%).

The signs and symptoms of anemia were not predictive of the iron status or the presence of iron deficiency anemia [Table 3].

The relation between food supplements and anemia, the status shows that food supplements like folic acid, iron, or iron-rich

| Table 1: Baseline characteristics of the study participants |
|-----------------------------------------------------------|
| Demographics (200 participants) when available |
| Age (years) |
| (19-27) |
| Mean: 20.4, |
| median: 22 |
| 25th, 75th percentiles (21-23) |
| City of living |
| Abha and other highland Aseer: 150 |
| Aseer lowland: 43 |
| other regions of SA: 7 |
| Nationality |
| Saudi: 199 |
| Non-Saudi: 1 |
| Marital status |
| Single: 190 |
| Married: 10 |
| Previous childbirth: 2 |
| Currently pregnant: 1 |
| History of anemia |
| Current or past history of iron deficiency anemia: 48 |
| G6PD: 4 |
| Sickle cell trait: 2 |
| Thalassemia minor: 2 |
| Family history of Anemia |
| Iron deficiency anemia: 44 |
| G6PD: 8 |
| SCD: 7 |
| Thalassemia: 3 |
| Other Medical history |
| Bronchial Asthma: 4 |
| PCOS: 2 |
| Hypothyroidism: 2 |
| SLE: 1 |
| Depression: 1 |
meals found to be significantly associated with anemia with Monte Carlo’s exact probability value (MC^2 P). For folic acid P value is 0.005, for iron P value is 0.047, and P value for iron-rich meals is 0.048, which are < 0.05 at 0.05 level of significance. The data interpret that the use of food supplements like folic acid, iron, or iron-rich foodstuffs among college girls is indicative of their anemia status [Table 4].

**Discussion**

The present study showed a high prevalence of iron deficiency status (ID) (63%, serum ferritin <20). Among these, 35% of the participants have normal hemoglobin (latent or early iron deficiency) and 17.5% have iron deficiency anemia (low iron store accompanied by Hb <13 g/dl). Although the prevalence is high, these figures might underestimate the true iron status level, given that 41 (20.5%) participants have hemoglobin <13 g/dL, and some references use higher serum ferritin cut-off (20–40 ng/mL) to define latent iron deficiency. Of note so far, there are no local studies to validate these cut-off values in our specific population living at high altitude. The present study used the WHO recommended definitions of anemia. Serum ferritin is thought to be independent of high altitude.

Table 2: Iron deficiency anemia (Hb<13) using a cut off value of serum ferritin < 15 and < 20. Serum ferritin < 20 seems more discriminatory. Only 3 students with Hb < 13 have ferritin > 20

| Ferritin (g/dL) | Ferritin c2 | Normal | Microcytosis |
|----------------|-------------|--------|--------------|
| <15            | 15+        | 4      | 2           |
| 15+            | 25         | 12.5%  | 45          |
| 13+            | 20         | 10.0%  | 69          |

| Ferritin (g/dL) | Ferritin c2 | Normal | Microcytosis |
|----------------|-------------|--------|--------------|
| <20            | 29          | 14.5%  | 9            |
| 20+            | 1           | 0.5%   | 2            |
| <20            | 30          | 15.0%  | 58           |
| 20+            | 15          | 7.5%   | 56           |

Table 3: Distribution of signs and symptoms according to anemia status among sampled students

| Signs/symptoms | IDA | Latent iron deficiency | Normal | P |
|----------------|-----|------------------------|--------|---|
| Recurrent headache | rare | 6 | 50.0% | 53 | 51.0% | 41 | 48.8% | 0.969 |
| | Sometimes | 6 | 50.0% | 48 | 46.2% | 40 | 47.6% |
| | Always | 0 | 0.0% | 3 | 2.9% | 3 | 3.6% |
| Feel fatique or tired | rare | 2 | 16.7% | 24 | 23.1% | 24 | 28.6% | 0.416 |
| | Sometimes | 7 | 58.3% | 65 | 62.5% | 53 | 63.1% |
| | Always | 3 | 25.0% | 15 | 14.4% | 7 | 8.3% |
| Have chronic diarrhea | rare | 7 | 58.3% | 86 | 82.7% | 70 | 83.3% | 0.179 |
| | Sometimes | 5 | 41.7% | 18 | 17.3% | 13 | 15.5% |
| | Always | 0 | 0.0% | 0 | 0.0% | 1 | 1.2% |
| Have recurrent nausea | rare | 7 | 58.3% | 73 | 70.2% | 64 | 76.2% | 0.516 |
| | Sometimes | 4 | 33.3% | 24 | 23.1% | 18 | 21.4% |
| | Always | 1 | 8.3% | 7 | 6.7% | 2 | 2.4% |
| Have palpititation \ chest pain | rare | 6 | 50.0% | 59 | 56.7% | 56 | 66.7% | 0.424 |
| | Sometimes | 6 | 50.0% | 44 | 42.3% | 26 | 31.0% |
| | Always | 0 | 0.0% | 1 | 1.0% | 2 | 2.4% |
| Experiencing fainting attacks \ lightheadness | rare | 8 | 66.7% | 63 | 60.6% | 65 | 77.4% | 0.130 |
| | Sometimes | 4 | 33.3% | 39 | 37.5% | 19 | 22.6% |
| | Always | 0 | 0.0% | 2 | 1.9% | 0 | 0.0% |
| Have breathlessness | rare | 8 | 66.7% | 57 | 54.8% | 58 | 69.0% | 0.132 |
| | Sometimes | 4 | 33.3% | 46 | 44.2% | 23 | 27.4% |
| | Always | 0 | 0.0% | 1 | 1.0% | 3 | 3.6% |

P: Pearson χ² test
The prevalence of IDA in our study (17.5%) is higher than reported in industrialized nations but lower than reported in developing countries. The results of the present study agreed with those reported by Alkindi S et al., (2018)[18] who reported that ID was common between the female students at Oman universities as 38% and 26% of students showed ID and IDA, respectively. There is no well-defined global geographic demarcation, but it can be estimated that most preschool children and pregnant females in nonindustrialized countries and at least 40% in industrialized countries suffer from IDA (WHO, 2011).[19]

In contrast with our study Shill et al. in 2014[20] reported that 55.3% of Bengali college students suffered from IDA, of whom 63.3% were female. Lee JO et al., (2014)[21] reported that IDA is more common in preschool children and population above 65 years old, females especially pregnant women, and provoked by poor socioeconomic status. Pandey S and Singh A, (2013)[22] reported that 52.7% of female medical students in Tehran, Iran had a normal iron status while only 4.0% suffered from IDA.

In our study, both personal and family history of iron deficiency anemia was associated with the presence of iron deficiency [Table 5]. This is unlikely to be due to hereditary diseases. Instead, this suggests that social and diet habits contribute to the development of iron deficiency. History of thalassemia and G6PD deficiency was also associated with iron deficiency. While G6PD deficiency is known to be associated with iron deficiency, if frequent bouts of intravascular episodes are developed, thalassemia is not usually associated with iron deficiency unless other causes, like blood loss or menorrhagia are causing coexistent iron deficiency.

The effect of high altitude on the iron stores and hematological parameters cannot be ignored (Cook JD et al., 2005).[23] The majority of our participants are nates from the highland Ascer region Southwestern Saudi Arabia and almost all are chronic dwellers. Although, we adjust hemoglobin cut-off for the high altitude, other RBC parameters seem to be also affected. For example, high altitude-related hypoxia-induced erythropoiesis is evident by the prevalent erythrocytosis among almost all participants [Table 6]. The prevalent microcytosis, with and without anemia (63.5%) could be an indicator of reduced iron stores. However, microcytosis is thought to be a late sign of iron deficiency and that hemoglobin drops before MCV does. It could be that subject may have had higher baseline hemoglobin, which may explain the presence of both erythrocytosis and microcytosis at higher than expected hemoglobin. It should be emphasized, though, that the normal range of MCV has not been validated for the high altitude, other RBC parameters seem to be also affected.

Figure 1: Prevalence of iron deficiency using Hb threshold of 13 g/dL and 12 g/dL.

Table 4: Relation between food supplementations and anemia status among sampled students

| Supplementation          | IDA Percentage | Latent iron deficiency Percentage | Normal Percentage | P   |
|--------------------------|----------------|----------------------------------|-------------------|-----|
| Use folic acid           | No             | 83.3%                            | 102               | 98.1%| 83   | 98.8%| 0.005*|
| USE YES                  |                |                                  |                   |     |      |      |      |
| Use Vit D                | No             | 83.3%                            | 87                | 83.7%| 73   | 86.9%| 0.813|
| USE YES                  |                |                                  |                   |     |      |      |      |
| Use iron                 | No             | 91.7%                            | 97                | 93.3%| 84   | 100.0%| 0.047*|
| USE YES                  |                |                                  |                   |     |      |      |      |
| Use omega 3              | No             | 100.0%                           | 103               | 99.0%| 83   | 98.8%| 0.926|
| USE YES                  |                |                                  |                   |     |      |      |      |
| Use Vit-B complex        | No             | 100.0%                           | 103               | 99.0%| 83   | 98.8%| 0.926|
| USE YES                  |                |                                  |                   |     |      |      |      |
| Use Ca                   | No             | 100.0%                           | 104               | 100.0%| 84 | 100.0%| -   |
| USE YES                  |                |                                  |                   |     |      |      |      |
| Use multi-Vit supplement | No             | 83.3%                            | 87                | 83.7%| 73   | 86.9%| 0.813|
| USE YES                  |                |                                  |                   |     |      |      |      |
| Have iron rich meals     | Not at all      | 33.3%                            | 30                | 28.8%| 27   | 32.1%| 0.048*|
| Always                   |                |                                  |                   |     |      |      |      |

*P: Mont Carlo exact probability, *P<0.05 (significant)
The effect of high altitude on the iron store has been studied before (Gassmann M and Muckenthaler MU 2015).[23] The hypoxia-induced erythropoiesis will utilize the available iron store until restricted by unavailability. The erythropoiesis induces the production of erythroferrone, which results in suppression of hepcidin to allow increased iron absorption and iron release from the store. The serum ferritin level is, however, thought not to be affected by high altitude (WHO, 2011).[24]

The present study showed that the symptoms of anemia like fatigue, headache, diarrhea, and nausea do not correlate with the presence of anemia. There was no significant statistical difference reported among the IDA status, ID symptoms, and normal conditions of college girls [Table 3]. This is not surprising knowing that iron deficiency is a slowly developing chronic illness that allows enough time for the body to adapt. Our results agree with those reported by (Lopez A., 2016)[25] and (Soppi ET, 2018)[26] who stated that weakness, poor concentration, and poor productivity are nonspecific symptoms caused by low oxygen delivery to body tissues and may be due to other causes like depression, celiac disease, multiple pregnancies, long duration of heavy menstruation, migraine, and fibromyalgia/chronic fatigue syndrome.

Iron deficiency state is associated with decreased cognitive and physical performance. Moreover, a deleterious effect in the developing embryo might result from prenatal anemia. A Swedish large population registry study found that the prevalence of autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), and intellectual disability (ID) were found to be higher among children born to mothers diagnosed with anemia within the first 30 weeks of pregnancy (4.9% ASD, 9.3% ADHD, and 3.1% ID) compared with mothers without anemia or those diagnosed later in pregnancy.[27]
Screening and iron supplementation and/or food fortification with iron in order to prevent iron deficiency has been studied. There is so far no strong evidence to support this practice. Although, the practice of supplementing pregnant ladies with iron seems common. The U.S. Preventive Services Task Force (USPSTF) 27 concluded that the evidence for screening and/or iron supplementation for pregnant women is insufficient. Nevertheless, the iron supplement seems effective in preventing anaemia in the young menstruating woman, and children younger than 12 years of age (Low MSY et al., 2016) and (De-Regil LM et al., 2011), although at the expense of gastrointestinal side effects. It is expected that the availability of safer intravenous iron formulation will make screening more rewarding and replacement easier.

Deciding which test to use for screening iron store status is important. The symptoms of anemia are notoriously nonspecific and would not be useful alone to predict the presence of iron deficiency or iron-deficiency anemia. The hemoglobin level does not seem to correlate well with the iron stores, especially at high altitudes. Serum ferritin is not affected by altitude but affected largely with infections and inflammations. Studies have explored other CBC parameters that can detect anemia early like reticulocytes, hemoglobin content, but these need to be validated for use as screening, especially at high altitude populations. 10

**Conclusion**

Iron deficiency (latent and IDA) is prevalent among female college students at the studied high altitude region. There is a real need to expand this study to other colleges and all vulnerable female (pregnant ladies and women of childbearing age). The traditional risk factors of iron deficiency were not shown to be implicated. More studies to explore dietary habits are needed. The primary care provider should maintain a high degree of suspicion in order to initiate lab screening as iron deficiency is frequently asymptomatic. The effect of high altitude on the iron store and hematological parameters need to be studied further. Moreover, a field survey, to determine the reference range of hematological parameters that are directed to our ethnic group, geographical area, and high altitude is needed. Symptoms of anemia and hemoglobin level are not good clinical correlates to suggest the presence of iron deficiency. Hence, we suggest implementing female-colleges wide screening at admission and biannually thereafter. In future, we are planning to study the association between iron status and overall college performance of the students.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate participants consent forms. The participants understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**

There are no conflicts of interest.

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