Iron Deficiency Status and Anaemia among Athletes in Osogbo, Nigeria

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Summary

BACKGROUND

Athletes are highly at risk of minimal iron consumption due to the nature of their diet which is mainly based on calorie consideration without regard for an iron-embedded diet. This study determined the iron deficiency status and anaemia in athletes across all sports in Osogbo, Nigeria.

MATERIALS AND METHODS

Seventy (70) subjects comprising 55 and 33 age-sex matched athletes and healthy non–athlete controls respectively, were recruited into this study from Osogbo City Stadium Nigeria. Parameters assessed include packed cell volume (PCV), haemoglobin concentration (Hb) and the red cell indices; serum iron; total iron-binding capacity; and ferritin.

RESULTS

There was a general and gender-specific significant reduction in the mean ±SD level of all haematological parameters ($p<0.05$) of the athletes compared with the controls with the TIBC, serum iron and ferritin reduced though statistically insignificant. Moderate anaemia was generally observed (64%) in the entire populace while those with severe anaemia, mainly the females (5%). Iron deficiency was present majorly in the female group with one male displaying iron depletion features.

CONCLUSION

Iron deficiency, depleted iron store and anaemia are well observed in the athletes with the females presenting more anaemic conditions than the male counterparts all of which result from their dietary constituents, non-supplementation of haemoglobin, mechanical induced intravascular haemolysis, uncompensated blood loss due to menstrual flow in females and exercise-induced inflammatory cytokine.

Keywords: *Iron deficiency, Athletes, Anaemia and Iron Depletion.*

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Introduction

Iron deficiency is the commonest nutritional predicament responsible for around 500-600 million cases of iron deficiency anaemia globally, even though it hardly causes death, it has a substantial negative effect on human health [1,2] Despite being easy to diagnose and treat in developed countries, it could still be ignored by medical practitioners sometimes; however, it is a major health concern affecting the majority of people in less developed countries as a result of poor socioeconomic status, poor nutrition and inadequate food supply [2]. Broadly speaking, the prevention and effective treatment of this disease remains poor globally [2].

Iron deficiency development occurs in three stages; the first stage depicts lower

serum ferritin levels due to the depletion of iron storage in reticuloendothelial cells of the liver, spleen and bone marrow, whereas no changes are usually observed in the haemoglobin levels [3, 4]. Therefore, medical practitioners who examine haemoglobin and haematocrit levels alone without evaluating serum ferritin levels misdiagnose athletes as having adequate or sufficient iron [3]. The second stage of iron deficiency occurs with the presence of reduced serum iron, transferrin saturation and increased total iron-binding capacity [3]. The first and second stages of iron deficiency are known as iron-deficient non-anaemia [4]. In the third stage, also known as the final stage of iron deficiency, haemoglobin formation is reduced as a result of inadequate iron supply which eventually results in anaemia [3, 4].

Athletes are at a higher risk of minimal iron consumption due to the nature of their lifestyle whereby their diet is mostly based on reduced-calorie food intake and eating disorders [5]. These include those engaged in wrestling, rowing, gymnastics dancing, nationwide sports and those who participate in endurance sports; they are believed to have an increased rate of iron turnover conditioned by losses which are caused as a result of excessive sweating, gastrointestinal haemorrhage, haematuria, haemolysis, intensive exercise, inadequate dietary consumption and absorption of iron [5]. Athletes have been observed to avoid specific types of food such as red meats and eggs that are generally recognized as the dietary sources of iron [3] which could contribute to the lower level of blood haemoglobin, haematocrit and ferritin values usually encountered in professional athletes [6]. The preliminary research that showed the low levels of the aforementioned haematological parameters in athletes led to the establishment of a theory called sports anaemia to explain this phenomenon [6].

The maximum performance of athletes mainly depends on the maintenance of iron levels which is achieved by consuming food rich in iron and its subsequent digestion [3]. The production of haemoglobin and the resultant ability of the body to carry oxygen from the lungs to other body tissues are compromised in athletic individuals with iron deficiency [3]. Furthermore, athletes who suffer from iron deficiency present with symptoms such as vomiting, recurrent infections, dyspnoea, diseases of the respiratory system, tiredness, lack of strength, and deficiency of energy and they usually become easily exhausted [3]. Historically, the high incidence of iron deficiency anaemia amongst athletes has been known to result from insufficient iron consumption in their diet and excessive iron depletion or both [5].

This study aimed to evaluate the effect of athletic training and career on iron status and haematological indices to educate athletes on the effect of iron deficiency and to develop preventive strategies that might aid the development and implementation of policy to enable athletes in Nigeria to have a healthy dietary lifestyle and at the same time enhance their performance.

Materials and Methods

Subject selection and study site

This was a community-based cross-sectional study using the convenience sampling method, which was conducted for a period of 6 months (September 2019-February 2020) in Osogbo, Nigeria. A total of seventy (70) subjects comprising 55 athletes (25 females and 30 males) and 30 non–athletes (15 females and 15 males) who served as controls, were recruited for this study. All participants were aged between 16-40 years, the athletes were recruited from Osogbo City Stadium, Osun State Sports Council Osogbo and Omo West Area Osogbo whilst the sex-age matched control group comprise of non-athletic healthy individuals recruited from the administrative staff of the Osogbo City Stadium and the Ladoke Akintola University of Technology, College of Health Sciences,

Osogbo, Nigeria. The study excluded subjects who did not meet the eligibility criteria which include smoking, post-menopausal stage in females and those with any medical or underlying illnesses. Athletes who participated in all kinds of sports were included in this study. Samples obtained were analyzed at the Medical Laboratory Science Research Laboratory, Isale-Osun, Osogbo, Osun State, Nigeria.

Ethical Approval

Ethical approval for the study was obtained from the Ethics Committee of the Ladoke Akintola University of Technology Teaching Hospital Osogbo, Osun State, Nigeria and permission was obtained from the Osogbo Sports Council, Osogbo, Osun State, Nigeria. The study information was provided to the potential participants and written informed consent was obtained from each participant.

Blood sample collection and storage

Exactly six (6) millilitres of venous blood were collected from each participant into Ethylene Diamine Tetra-acetic acid (EDTA) and plain bottles. The blood in the plain bottles was allowed to clot and centrifuged at 2,000 - 3,000 rpm for 20 minutes. The supernatant serum was aspirated into the cryo-vial tube and stored at -20°C until subsequent analysis.

Haematological analysis using automated machine

The Sysmex KX-21N (Japan) autoanalyser was used to measure the packed cell volume (PCV), haemoglobin concentration (Hb), mean cell volume (MCV), and mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC).

Estimation of serum iron

using the Ferrozine method as described by Suwansaksri *et al.*, (2003) [7] and Carpenter *et al*. (2017) [8] with the Ferrozine kit (Chemelex Rgt, S.A.).

The colorimeter was adjusted to zero with distilled water, and 1.0 ml of working reagent was dispensed into a cuvette. One drop of R3 (Ferrozine) was dispensed into working reagent blank, calibrator and sample test tubes. Two hundred microliters of distilled water were dispensed into each tube and sample blank tube. It was mixed and incubated at 37°C for 5 minutes. The absorbance of the calibrator and sample was read at 562nm against the working reagent blank.

Estimation of total iron-binding capacity (TIBC)

We used the Spectrophotometric method9. Five hundred microliter (500ul) of sample and 1ml of R5 saturating solution was dispensed into tubes, well mixed and incubated for 10 minutes at room temperature. To each tube, an R6 precipitating agent (3 spoons) was added, mixed and incubated for 10 minutes at room temperature. Then the solution was centrifuged for 15 minutes at 3000 r.p.m. The supernatants were carefully collected and the concentration of iron was measured spectrophotometrically at 525nm wavelength.

Estimation of human ferritin

We used the Enzyme-Linked Immunosorbent (ELISA) Assay Technique [10, 11]. Ferritin levels were determined using the SL0702Hu-Human Ferritin ELISA kit (SunLong Biotech co., Ltd. Hangzhou, China) according to the manufacturer's instructions. This assay is based on the double-antibody Sandwich enzyme-linked immunosorbent assay (ELISA) technique. The Microelisa strip plate has been pre-coated with an antibody specific to ferritin (FE). Standards or samples are added to the appropriate Microelisa strip plate wells and combined with the specific antibody, then a Horseradish Peroxidase (HRP)-conjugated antibody specific for FE is added to each microelisa strip plate well and incubated. Free components are washed away, and the TMB substrate solution is added to each well. Only those wells that contain FE and

HRP conjugated FE antibodies will appear blue in colour and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of FE.

For the purpose of categorization in this study, anaemia was grouped into moderate and severe conditions as haemoglobin concentration / PCV values of 10-11.9g/dl/ 30-32% and <10.0g/dL / <28% respectively while normal haemoglobin and PCV are >12g/dl and 33% [12,13] ; depleted iron store as serum ferritin <15ng/ml[14,15] ; iron deficiency as any two of TIBC>400ug/dl, MCV <70fl, serum iron <60ug/dl or serum ferritin <15ng/dl [15,16] ; and iron deficiency anaemia was detected and separated based on reduced haemoglobin (<10.0g/dL) coupled with two or more of; MCV <70fl, MCH(<20pg) and serum ferritin (<15ng/ml) [12,13].

Statistical analyses

All data were expressed as mean +/- SEM (Standard Error of Mean). Significant differences were tested using a student t-test using IBM SPSS version 25. A value of $p \leq 0.05$ was considered statistically significant.

Results

The mean ±SD level in the participants displayed a significant difference ($p<0.05$) between the Hb, PCV, MCV, MCH, and MCHC of the athletes and the controls with the athletes possessing reduced levels of these parameters whereas the TIBC, serum iron and ferritin had no statistically significant difference in comparison as depicted in Table 1($p<0.05$), the same pattern of occurrence observable across the gender (Figure 1) when compared with corresponding controls.

Figure 2 displays the distribution of athletes based on their anaemia status, being grouped for the severity of the condition using haemoglobin values.

Table 1:
The Mean Values of all Parameters Assessed in the Athletes and Control Subjects.

Parameter	Group	*Mean*	*Std. Deviation*	*F*	*p-value*
Hb (g/dL)	Athletes	10.825	1.1015	21.648	0.001*
	Control	13.4	1.545		
PCV (%)	Athletes	35.74	2.999	13.7755	0.001*
	Control	41.68	4.3825		
MCV (fL)	Athletes	68.03	4.1	18.5105	0.007*
	Control	75.035	4.187		
MCH (pg)	Athletes	20.585	1.9295	20.6015	0.001*
	Control	24.23	1.551		
MCHC (g/L)	Athletes	172.04	16.58	2.891	0.013*
	Control	185.11	4.2475		
Serum Iron (µg/dL)	Athletes	86.58	39.24	0.4435	0.3245
	Control	84.52	24.935		
TIBC (µg/dL)	Athletes	273.83	56.025	15.876	0.2155
	Control	294.6	81.91		
Serum Ferritin (ng/ml)	Athletes	36.28	21.96	0.6775	0.607
	Control	42.47	16.69		

***represent significant at p<0.05**
Legend: Hb- Haemoglobin; PCV- packed cell volume; MCV- Mean Cell Volume; MCH- Mean Cell Haemoglobin; MCHC- Mean cell haemoglobin concentration; TIBC- Total Iron binding capacity.

The participants with normal haemoglobin concentration are 31% (17) of the general athlete population; those with moderate anaemia are the highest in the population with 64% (35) while those with the severe anaemic condition are the least with 5% (3) distribution. The athlete population with normal Hb/PCV, moderate and severe anaemia comprises 12, 17 and 1 male as well as 5, 1 and 2 female participants respectively (Table 2) depicting female participants having higher severe anaemia than their male counterparts. One male participant with an iron depletion factor was observed in the study and the female athletes also had higher iron deficiency features than the male with overall 32.7% participants in such category. Exactly 17 participants of the 55 athletic participants have iron deficiency anaemia with higher possession among the females (10) than the male (7) participants (Table 2).

Table 2:
Distribution of Iron-deficiency Anemia (IDA) Associated Factors among the Athletes.

	Factors	Male (n-30)	Female (n-25)	All (n-55)
Anaemia (Hb g/dl; PCV %)	Normal (12g/dl; 33%)	12 (40%)	5 (20%)	17 (30.9%)
	Moderate (10-11.9g/dl; 30-32%)	17(56.7%)	18 (72%)	35 (63.6%)
	Severe Anaemia (<10g/dl; <28%)	1 (3.3%)	2 (8%)	3 (5%)
Depleted Iron store		1 (3.3%)	-	1 (1.86%)
Iron deficiency		8 (26%)	10 (40%)	18 (32.7%)
Iron deficiency Anaemia (IDA)		7 (23%)	10 (40%)	17 (30.9%)

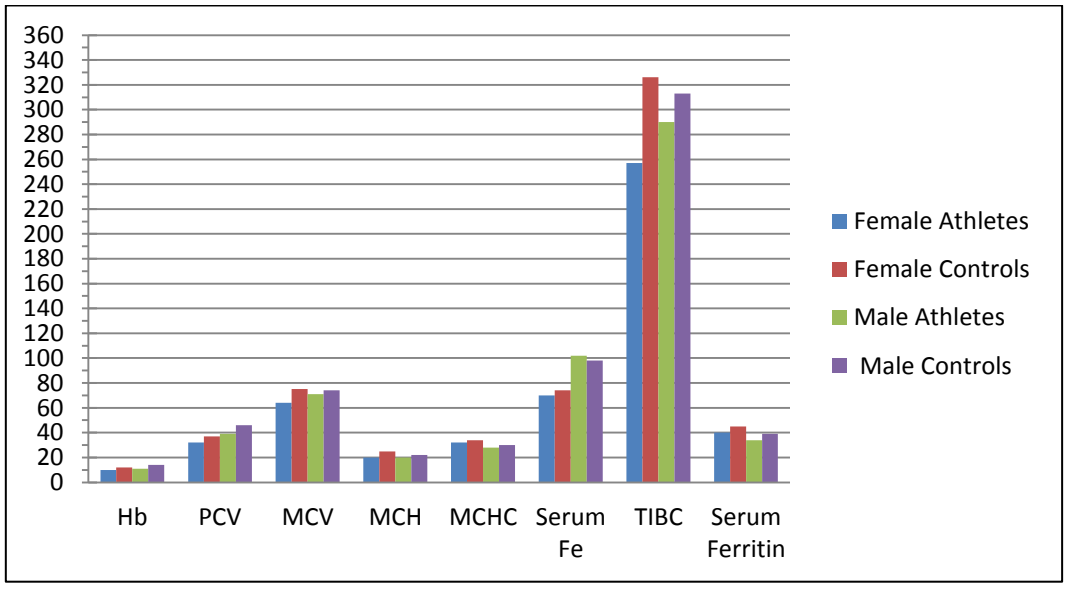

Figure 1:
The Mean Levels of Anaemia Indicators among the Athletes Based on the Gender
Legend: Hb- Haemoglobin; PCV- packed cell volume; MCV- Mean Cell Volume; MCH- Mean Cell Haemoglobin, MCHC- Mean cell haemoglobin concentration; Fe- Iron; TIBC- Total Iron binding capacity.

Discussion

The global prevalence of anaemia has been estimated as 24% with iron deficiency anaemia contributing about 50% of the condition and a higher rate presented in developing countries especially the western sub-Saharan region [17], [18], [19], [20]. A report on the incidence of iron deficiency in elite male athletes indicated a 43% anaemia occurrence and 32% in female athletes generally [21,22] hence signifying the existence of the condition even in athletes.

In this study, the reduced value of the major indicators of anaemia which are Hb, PCV and the red cell indices observed across the athletic participant both generally and across the genders when compared with the various non-athletic controls are indicative of anaemia which is deduced to likely result from increased plasma volume leading to sports anaemia [23]; the intravascular haemolysis of most senescent red blood cells, that ensued from physically induced destruction of the red cells while being forced through capillaries of contracting muscles [23]; and the microcytosis and hypochromia from the reduced absolute values are the possibility of insufficient compensatory diet for the various losses of red cell components due to the dietary characteristics of the athletes. Several authors in similar studies have reported the same pattern of results in athletes across different types of aerobic and anaerobic exercises both among the male and female population [4,24,25,26].

Also, 5% of the athlete populations are severely anaemic while the majority of them are moderately anaemic with more preponderance in the female anaemic cluster which further emphasizes the hemolytic effects and lifestyle impact of the exercise on the blood cells of the individuals. The implication for the female majority is due to inadequate compensatory renewal of blood loss from the menstrual cycle along with their lackadaisical attitude to the use of dietary supplements as being advocated for by researchers [4] hence the continued reduction in anaemia indicators.

Furthermore, a male anaemic athlete was observed to present with depleted iron storage as serum ferritin was <15ug/ml displaying a consequence of a long dietary habit of iron-lacking meals coupled with vigorous exercise and a possible iron absorption disorder all of which results in reduced iron stores and anaemia.

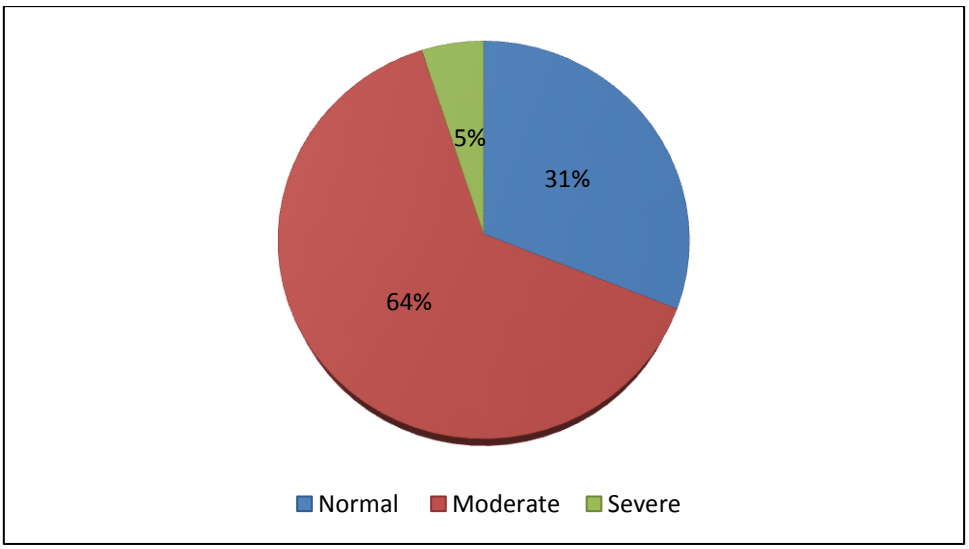

Figure 2:
Distribution of Anaemia Status According to Haemoglobin Level Among the Athletes

This development emphasizes the need to increase iron-containing food in the meal of athletes despite the high calories in their diet as iron depletion is even observed in supposed healthy individuals who are blood donors [27].

Also, the majority of those with iron deficiency were observed to have iron deficiency anaemia which implies their anaemia is majorly due to iron deficiency. This development affirms that the iron-deficient nature of their food coupled with the hemolytic effect of their exercise activities as well as exercise-induced inflammatory cytokine production can all result in lower aerobic fitness as observed by Tsai *et al.*, (2019) [28]. Tsai and colleagues reported that a positive correlation was discovered to exist between the overall capacity of haemoglobin and aerobic fitness, with no observable association existing with anaerobic fitness; this is because a 2-minute anaerobic exercise does not require oxygen due to the actions of intramuscular ATP, creatinine phosphate and lactate which are required in the first 30 and 90 seconds respectively for energy consumption [28, 29].

In addition, some athletes with moderate anaemia were not found within the iron-deficiency anaemia group which implies their anaemia is not occurring due to iron deficiency but may result from other causes such as megaloblastic anaemia. This occurrence still emphasizes diets lacking in vitamins as obtained in some high-calorie energy-giving diets athletes are placed on. However, this study could not ascertain the basis of anaemia in such individuals hence the need for further studies that investigate all causes of anaemia in athletes.

Limitation of the study

The participants in different sporting activities were not separated due to their uneven and irregularly distribution to relate a kind of sport with the anaemic state of a participant.

Conclusion

This study has shown there is reduced haemoglobin concentration which invariably indicates anaemia; depleted iron store; iron deficiency; and iron deficiency anaemia in the studied athletes with the females presenting more anaemic conditions than the males all of which result from their dietary constituents, non-supplementation of haemoglobin, mechanical induced intravascular haemolysis, uncompensated blood loss due to menstrual flow in females and exercise-induced inflammatory cytokine. This calls for advocacy and sensitization to athletes on the need to ensure balance in their diet with iron supplementation to avoid the detrimental effects of iron deficiency and anaemia.

Acknowledgement

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Conflict of interest

There is no conflict of interest associated with this study.

Author contributions

All authors contributed significantly to the study. Bamisaye E.O. and Akanni E.O. designed, computed analyses and reported the study. Salau O.A, Adebayo S. R and Adebayo S. collected the samples and performed the analysis.

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