EVALUATION OF PLASMA LEVELS OF INTERLEUKIN 6 AND IRON STATUS OF FOOTBALLERS IN A NIGERIAN UNIVERSITY

Comment [ZK1]: Football players

ABSTRACT

To determine the levels of interleukin 6 (IL-6) and iron status of footballers in Madonna University, Elele, Rivers State, Nigeria. A total number of 100 subjects were recruited for the study, comprising of 50 subjects before playing football (25 males and 25 females) and 50 subjects after playing football (25 males, 25 females) from Madonna University Nigeria, Elele Campus, Rivers State, Nigeria. The data obtained from the study were presented as Mean ± SD in tables and analysed using student t-test for parametric data using SPSS version 20. The level of significance was set at $p<0.05$. The results showed significant increase ($p=0.004$) in interleukin 6 (IL-6) of footballers after playing compared to it before playing and no significant change ($p=0.505$) in the iron level of footballers after playing compared to it before playing respectively. The results also showed no significant change in interleukin 6 compared among footballers based on gender and age groups and no significant change in the iron level of all the subjects. The study showed an increase in interleukin 6 (IL-6) of the footballers after playing compared to the level before playing which shows that the physical activity increases the level of interleukin 6 and but has no effect on the iron level after football game.

Comment [ZK2]: Football players

Comment [ZK3]: Any time lapses present?

***Keywords**: interleukin 6, iron, football, exercise, students, University*

INTRODUCTION

It has been reported that Physical exercise like football there was a lower load to muscle to perform contraction (Moreira *et al.*, 2014). Muscle adapted to physical load by secreting interleukin-6 into blood stream. Interleukin-6 is an important myokine for muscle adaptation during sports especially football (Chowdhury *et al*., 2020). It is responsible for inflammatory regulation, protein synthesis, lipid deposition, metabolism and muscle development. Interluekin-6 was also related to iron deposition involving ferritin, hepcidin and haemoglobin (Nakagawa *et al*., 2014).

Comment [ZK4]: Grammar check

Comment [ZK5]: Load of what?

Interleukin-6 is a pro-inflammatory cytokine that can increase following physical exercise (White *et al.,* 2020). Higher level of interleukin-6 is linked to high response of inflammation due to physical exercise like in football which involves the entire body (Cabral-Santos et al 2015). Interleukin-6 stimulates synthesis of hepcidin so that its level raised in the blood during inflammation (Nemeth *et al*., 2004; D'Angelo, 2013). It is reported by Cullen *et al.* (2016) that the effect of exercise intensity and volume on the interleukin-6 response increases in the high intensity group compared to the low intensity group.

Interleukin 6 (IL-6) is a cytokine that plays a role in the specific antigen immune response and acute inflammatory response (Wolf *et al*., 2014, Obeagu *et al.,* 2019; Ifeanyi *et al.,* 2020). It is produced in several types of cells and can act in a large number of tissues (Hirano *et al.,* 1990). IL-6 plays a crucial role in the defense response and has a pleiotropic characteristic that can determine more than one phenotypic characteristic (Hirano *et al.,* 1990; Kang *et al.,* 2020). When moderate to extreme intensity exercise (>85–90% of maximal heart rate) is performed, the IL-6 level in the blood circulation increases. Skeletal muscle contraction is the stimulus for its release; thus, it is considered a myokine as it is produced, expressed, and released by muscle and has paracrine and endocrine effects (Pedersen *et al.,* 2004; Reihmane and Dela, 2014). A reduction in the availability of carbohydrates for exercise stimulates the release of IL-6 as it can assist in the maintenance of serum glucose levels during exercise (Reihmane and Dela, 2014). IL-6 is an important marker since an increase in its concentration is associated with an increase in the levels of acute-phase inflammatory proteins, such as C-reactive protein (Estrela *et al.,* 2017), the risk of cardiovascular events, and the process of rupture (Zhao *et al.,* 2017).

Hepcidin plays a key role of ferroportin opening and iron transport via membrane regulation (Coates, 2014). Hepcidin inhibits ferroportin opening so that iron fail to export across membrane of erythrocyte and macrophage (Ganz and Nemeth, 2012).

The role of haeme and nonhaeme iron in biological function and sports has been clarified via human and animal studies, and several classic reviews have been published (Finch and Huebers, 1982; Dallman, 1982) and updated (Azevedo *et al.,* 1989). Not surprisingly, haemoglobin iron, when lacking, can greatly affect sports through a reduction in oxygen transport to exercising muscle. Endurance performance at reduced exercise intensities, however, is more closely related to tissue iron concentrations because of the strong association between the ability to maintain prolonged submaximal exercise and the activity of iron-dependent oxidative enzymes.

To determine the levels of interleukin 6 (IL-6) and iron status of footballers in Madonna University, Elele, Rivers State, Nigeria

Comment [ZK6]: Conjunction misuse

Comment [ZK7]:

Comment [ZK8]: Grammar check

Comment [ZK9]: The purpose of the study is not connected with the introduction part, there is a lack of idea fluency in the introduction

Comment [ZK10]: The introduction part is lacking fluency of information

MATERIALS AND METHODS

Study Design

The project is a cross-sectional study involving subjects recruited from football players of Madonna University Nigeria, Elele Campus. The subjects encompass males and females football players and apparently healthy individuals age and sex-matched as the controls. The study is a quantitative research to assess the levels of interleukin 6 and iron status of the football players among the students of the University.

Study area

The research was carried out on football players in Madonna University Nigeria, Elele Campus, Rivers State, Nigeria. It is located in the South South part of Nigeria.

Study population

A total number of 100 subjects were recruited for the study, comprising of 50 subjects before playing football (25 males and 25 females) and 50 subjects after playing football (25 males, 25 females) from Madonna University Nigeria, Elele Campus, Rivers State, Nigeria. They all gave consent to participate in this study.

Comment [ZK11]: What's time determinant used in the current study?

Inclusion criteria

Students of Madonna University Nigeria, Elele Campus that are footballers without any sign of disease and apparently healthy individuals were selected for the study.

Comment [ZK12]: Can you explain in details more about subjects' age, healthy status? How did you evaluate the healthy status of recruiters?

Exclusion criteria

Any Student of Madonna University Nigeria, Elele Campus that is sick or showed s any sign of disease, pregnant, smoker, alcoholics or aged were excluded for the study.

Comment [ZK13]: Which age has been excluded from the current study?

Procurement of iron

A commercially prepared serum iron test kit product of BioSystems reagents and instruments company limited were used to assay the iron level.

Ethical consideration

The approval for the study was obtained from the Department of Medical Laboratory Science, Madonna University Nigeria, Elele Campus, Rivers State.

Laboratory Investigations

Interleukin 6 (IL-6) determination using Elabscience (Catalog No: E-EL-H0102)

Comment [ZK14]: Grammar error

Procedure

1. 100µL standard or sample was added to the wells and incubated for 90 min at 37°C
2. The liquid was discarded, immediately added 100µL Biotinylated Detection Ab working solution to each well and incubated for 60 min at 37°C.
3. The plate was aspirated and washed for 3 times
4. 100µL HRP conjugate working solution was added, incubated for 30 min at 37°C and aspirate d and washed the plate for 5 times
5. 90µL Substrate Reagent was added and incubated for 15 min at 37°C
6. 50µL Stop Solution was added
7. The plate was read at 450nm immediately and the results calculated.

Statistical analysis

The data obtained from the study were presented as Mean ± SD in tables and analysed using student t-test for parametric data using SPSS version 20. The level of significance was set at $p<0.05$.

RESULTS

Table 1: Mean ± SD values of interleukin 6 (IL-6) and Iron status of the subjects before and after playing football

Parameters	Before Playing	After Playing	t-value	P-value
IL-6 (pg/ml)	11.55±2.28	19.81±3.60	4.291	0.004*
Iron (ug/dl)	82.70±18.92	93.23±26.02	0.703	0.505

Comment [ZK15]: Can you explain the type of your samples?

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Comment [ZK17]:

Comment [ZK18]: Complete name of it?

Comment [ZK19]: What are your normal values? No text information is explained

Table 1 showed significant increase (p=0.004) in interleukin 6 (IL-6) footballers after playing (19.81±3.60 pg/ml) compared to before playing (11.55±2.28 pg/ml) and no significant change (p=0.505) in iron of footballers after playing (93.23±26.02 ug/dl) compared to before playing (82.70±18.92 ug/dl) respectively.

Comment [ZK20]: This statement should proceed the table.

Table 2: Mean ± SD values of interleukin 6 (IL-6) and Iron status of the Football players based on sex

Parameters	**Male**	**Female**	**t-value**	**P-value**
IL-6 (**pg/ml**)	17.99±2.43	18.86±6.53	-0.177	0.876
Iron (**ug/dl**)	95.20±36.49	81.95±10.40	0.494	0.670

Table 2 showed no significant change (p=0.876) in interleukin 6 (IL-6) of male footballers (17.99±2.43 pg/ml) compared to female footballers (18.86±6.53 pg/ml) and no significant change (p=0.670) in iron of male footballers (95.20±36.49 ug/dl) compared to female footballers (81.95±10.40 ug/dl) respectively.

Comment [ZK21]: The unite is confusing whether it's a micro or nano?

Comment [ZK22]: Same for this statement, it should proceed your table of results

Table 3: Mean ± SD values of interleukin 6 (IL-6) and Iron status of the Football players based on age bracket

Parameters	Below 20 years	21-30 Years	**t-value**	**P-value**
IL-6 (**pg/ml**)	13.19±4.36	18.86±6.53	-1.021	0.414
Iron (**ug/dl**)	90.05±43.77	81.95±10.40	0.255	0.823

Comment [ZK23]: Can you mention your included age group, like between 15-20 years or …?

Table 3 showed no significant change (p=0.414) in interleukin 6 (IL-6) of footballers aged below 20 years (13.19±4.36 pg/ml) compared to footballers aged above 21- 30 years (18.86±6.53 pg/ml) and no significant change (p=0.823) in iron of footballers aged below 20 years (90.05±43.77 ug/dl) compared to footballers aged 21- 30 years (81.95±10.40 ug/dl) respectively.

Comment [ZK24]: The statement is relocated before the table of information

DISCUSSION

The study showed increase in interleukin 6 (IL-6) of footballers after playing that was statistically significant. It is also known that muscular exercise enhances plasma levels of some cytokines (Ronsen *et al.,* 2002). Several studies demonstrated that strenuous exercise is accompanied by an increase in circulating pro-inflammatory responsive cytokines along with other bioactive stress molecules having some similarities with the response to sepsis and trauma (Hoffman-Goetz and Pedersen, 1994; Pedersen *et al.*, 1997). It has been shown that physical activity such as exercises to the muscles increase the level of secretion and release of interleukin 6 form the muscles as well as from the lymphocytes. Despite the difficulties inherent in measuring plasma cytokines concentrations (Ruiz-Argüelles, 1995), studies of subjects exercising intensively reported conflicting results. Some authors reporting increase (Ostrowski *et al.,* 1998) and others no changes (Rivier *et al.,* 1994) in IL-6 production after strenuous exercise. The stress and oxidation may increase the inflammatory process that will raise the levels of interleukin 6 and regulate iron production through hepcidin regulation. This study also shows a significant increase in IL-6 concentrations for footballers after playing. Thus, it has been demonstrated that plasma concentrations of IL-6 increases up to more than 100-fold during prolonged muscular exercise (Pedersen *et al.,* 2001). The augmented IL-6 plasma concentrations following football was

Comment [ZK25]: In muscles

Comment [ZK26]: In lymphocyte

Comment [ZK27]: Old reference, now days techniques for measuring cytokines are cheap and available.

Comment [ZK28]: Grammar check

associated with muscle damage in an earlier study (Pedersen *et al.*, 1998), but today it is very clear that exercise without any muscle damage also induces marked production of IL-6 and that IL-6 is produced as a direct consequence of contraction per se (Pedersen *et al.*, 2001).

When interleukin 6 was compared among the males and females, there was no significant change. This shows that gender has no effect on the levels of interleukin 6 as well as the iron status of footballers. Also, when interleukin 6 (IL-6) and iron were compared based on age groups such as below 20 years and 21-30 years of the footballers, there were no significant changes among the age groups.

Comment [ZK29]: Any supportive studies for these results? I think gender and age are important factors in cytokines changes

Conclusion

The study showed increase in interleukin 6 (IL-6) of the footballers after playing compared to the level before playing which shows that the physical activity increases the level of interleukin 6 and but has no effect on the iron level after football game.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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