EVALUATION OF PLASMA LEVELS OF INTERLEUKIN 6 AND IRON STATUS OF VOLLEYBALL PLAYERS IN A UNIVERSITY IN NIGERIA

ABSTRACT

To determine the levels of interleukin 6 (IL-6) and iron status of volleyball players in Madonna University, Elele, Rivers State, Nigeria. A total number of 80 subjects were recruited for the study, comprising of 40 subjects before playing volleyball (20 males and 20 females) and 40 subjects after playing volleyball (20 males, 20 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 of table 1 showed that there was increase ($p=0.003$) in interleukin 6 (IL-6) after playing volleyball compared to before playing volleyball and no significant change (0.079) in iron after playing volleyball compared to before playing volleyball. Table 2 showed that there was no significant increase ($p=0.164$) in interleukin 6 (IL-6) of males compared to females and no significant change ($p=0.589$) in iron of males compared to females. Table 3 showed that there was no significant increase ($p=0.921$) in interleukin 6 (IL-6) of volleyball players aged 15-25 Years compared to volleyball players aged 26-35 Years and no significant change ($p=0.503$) in iron of volleyball players aged 15-25 Years compared to volleyball players aged 26-35 Years respectively. The study showed increase in interleukin 6 (IL-6) of the volleyball players 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 volleyball game.

***Keywords**: Interleukin 6, iron, volleyball, inflammation*

INTRODUCTION

It has been reported that Physical exercise like volleyball 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 volleyball (Chowdhury *et al*., 2020). It is responsible for inflammatory regulation, protein synthesis, lipid deposition, metabolism and muscle development. Interluekin-6 (Replace with word It) was also related to iron deposition involving ferritin, hepcidin and haemoglobin (Nakagawa *et al*., 2014).

It is shown that 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 sports like in volleyball which involves the entire body (Cabral-Santos *et al*., 2015). Interleukin-6 stimulates synthesis of hepcidin so that its level increased 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 sports (>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. The stress on the muscles and lymphocytes together with monocytes may change the levels of interleukin 6 and iron levels after volleyball game and becomes necessary to carry out this research to ascertain what happens in the players the variables will affect the quality of life and wellbeing of the volleyball players.

To determine the levels of interleukin 6 (IL-6) and iron status of volleyball players in Madonna University, Elele, Rivers State, Nigeria (This is objective of work not introduction)

MATERIALS AND METHODS

Study Design

The project is a cross-sectional study involving subjects recruited from volleyball players of Madonna University Nigeria, Elele Campus. The subjects encompass males and females football players 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 volleyball 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 80 subjects were recruited for the study, comprising of 40 subjects before playing volleyball (20 males and 20 females) and 40 subjects after playing volleyball (20 males, 20 females) from Madonna University Nigeria, Elele Campus, Rivers State, Nigeria. They all gave consent to participate in this study.

Inclusion criteria

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

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.

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)

Assay procedure

100µL standard or sample was added to the wells and incubated for 90 min at 37°C

The liquid was discarded, immediately added 100µL Biotinylated Detection Ab working solution to each well and incubated for 60 min at 37°C. The plate was aspirated and washed for 3 times. 100µL HRP conjugate working solution was added, incubated for 30 min at 37°C and aspirate d and washed the plate for 5 times. 90µL Substrate Reagent was added and incubated for 15 min at 37°C. 50µL Stop Solution was added. 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 volleyball

Parameters	Before	After	t-value	*P-value*

IL-6 (**pg/ml**)	14.32±2.12	23.76±4.40	-4.321	0.003*
Iron (**ug/dl**)	75.04±9.10	92.32±16.90	-2.014	0.079

Table 1 showed that there was significant increase (p=0.003) in interleukin 6 (IL-6) after playing volleyball (23.76±4.40 pg/ml) compared to before playing volleyball (14.32±2.12 pg/ml) and no significant change (0.079) in iron after playing volleyball (92.32±16.90 ug/dl) compared to before playing volleyball (75.04±9.10 ug/dl)

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

Parameters	**Male**	**Female**	**t-value**	**P-value**
IL-6 (**pg/ml**)	20.26±3.26	26.10±3.60	-1.833	0.164
Iron (**ug/dl**)	98.40±2.97	88.27±22.47	0.602	0.589

Table 2 showed that there was no significant increase (p=0.164) in interleukin 6 (IL-6) of males (20.26±3.26 pg/ml) compared to females (26.10±3.60 pg/ml) and no significant change in iron (p=0.589) of males (98.40±2.97 ug/dl) compared to females (88.27±22.47 ug/dl) respectively.

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

Parameters	**15-25 Years**	**26-35 Years**	**t-value**	**P-value**
IL-6 (**pg/ml**)	23.56±6.17	24.06±1.03	-0.108	0.921
Iron (**ug/dl**)	87.37±19.23	99.75±14.78	-0.759	0.503

Table 3 showed that there was no significant increase (p=0.921) in interleukin 6 (IL-6) of volleyball players aged 15-25 Years (23.56±6.17 pg/ml) compared to volleyball players aged 26-35 Years (24.06±1.03 pg/ml) and no significant change in iron (p=0.503) of volleyball

players aged 15-25 Years (87.37±19.23 ug/dl) compared to volleyball players aged 26-35 Years (99.75±14.78 ug/dl) respectively.

DISCUSSION

The results of table 1 showed that there was increase in interleukin 6 (IL-6) after playing volleyball compared to before playing volleyball and no significant change in iron after playing volleyball compared to before playing volleyball.

Table 2 showed that there was no significant increase in interleukin 6 (IL-6) of males compared to females and no significant change in iron of males compared to females. The study showed increase in interleukin 6 (IL-6) of volleyball players 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 tedious sports 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 volleyball players 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 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).

Table 3 showed that there was no significant increase in interleukin 6 (IL-6) of volleyball players aged 15-25 years compared to volleyball players aged 26-35 Years and no significant

change in iron of volleyball players aged 15-25 Years compared to volleyball players aged 26-35 years respectively.

Conclusion

The study showed increase in interleukin 6 (IL-6) of the volleyball players 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 volleyball game.

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