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Effect of ageing on some biophysical parameters amongst males in Benin City, Nigeria

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ABSTRACT

This study was designed to assess the effect of ageing on the $CD4^+$ T cells count, testosterone, progesterone, and estrogen concentration in a view to evaluating the alterations associated with it. A total of 102 subjects aged between 20 and 80 years participated in the study.$CD4^+$ T cell was estimated using Partec cyflow counter. Testosterone, progesterone and estrogen were determined by enzyme-linked immunosorbent assay methods. Our finding revealed a significant decrease in absolute lymphocyte count, $CD4^+$ T cells count, progesterone, and testosterone concentration of the elderly males when compared with the young males ($P < 0.05$). Ageing induces a decrease in $CD4^+$ T cells, progesterone and testosterone level which could be linked to the complications associated it.

Keywords: CD4+ T cells, ageing, testosterone, hypogonadism, thymus gland.

INTRODUCTION

It is well-established that ageing is associated with a decline in immune function (Gyubeck and Wick, 2002). A major contributing factor to impaired cellular immunity in the elderly is a decline in T cell repertoire diversity. Thymic involution with age results in the export of fewer naïve T cells to the periphery. Decline in $CD8^+$ T cell repertoire diversity has been clearly associated with impaired responses to new infections (Yager et al., 2008; Cian et al., 2010; Rudd et al., 2011). A major age-associated defect for $CD4^+$ T cells has been shown to reduce Interleukin-2 production (Haynes and Lefebvre, 2011). The higher incidence of infectious diseases (influenza), autoimmunity (rheumatoid arthritis), cancer (prostate) and cardiovascular diseases is an obvious issue in ageing (Pawelec, 2007). The most important contributor to the decline in immune function in the elderly is the changes observed in adaptive immunity (Goronzy and Weyand, 2005). Telomeric repeats (TTAGGG) shortening that is associated with ageing has been linked with increased DNA mismatch (Di-Mitri et al., 2011).

Testosterone deficiency is common as men grow older (Harman et al, 2001).Testosterone levels peak between ages

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20 and 40 years, and then decline gradually by approximately 1% per year. Lower testosterone levels is associated with frailty, reduced sexual activity, insulin resistance, cognitive decline, cardiovascular events and mortality in ageing males (Rudd et al., 2011). Does ageing in males have any effect on the $CD4^+$ T cells and testosterone, progesterone and estrogen level? This study was designed to assess the effect of ageing on these biophysical parameters amongst males in Benin City, Nigeria with a view to evaluating the alteration associated with it.

MATERIALS AND METHODS

Subjects

A total of 102 subjects aged between 20 and 80 years participated in the study. The participants were randomly recruited. Obese or underweight (BMI between 20 and 30 kg/m^3), smokers, alcoholics, diabetic, cardiac, renal, and respiratory disease patients were excluded. The participants gave informed consent. 6 ml of venous blood was taken from the antecubital vein by venapunture. It was equally shared into an ethylene diamine tetra acetic acid container for absolute lymphocyte estimation and $CD4^+$ T cell count. The other portion was added into an anticoagulant free container and allowed clot. It was subsequently centrifuge at 750 x g for 15 minutes to obtain serum. The serum was immediately aliquoted into an eppendorf tube place on ice and immediately stored at -80 °C until testosterone, estrogen and progesterone were estimated.

Absolute lymphocyte count estimation

Absolute lymphocyte was determined using the sysmex automated Hematology analyzer as previously described by Ehiaghe et al. (2014).

$CD4^+$ T cell count estimation

$CD4^+$ T cell count was estimated using Partec cyflow counter as described by Partec cyflow counter (2006).

Serum testosterone estimation

Serum testosterone was determined by enzyme linked immune sorbent assay technique. This test kit operates on the basis of competition between the conjugates and the testosterone in the sample(s) for a limited number of binding sites on the antibody coated wells. Twenty-five micro liters of standard or sample(s) was added per microplate.100 µl testosterone hormone conjugate was added to the standard or sample(s) and covered with a sealing tape. It was incubated at 37 °C for 1 hour. The solution was discarded and microplates washed three times with 300 µl of 1X wash solution. 100 µl of tetramethylbenzidine one step substrate was added to each well and incubated for 15 minutes at room temperature in the dark with gentle shaking.100 µl of stop solution was added to each microplate. The intensity of the color developed was measured at 450 nm.

Serum 17β estradiol estimation

Serum 17β estradiol was determined by enzyme linked immune sorbent assay technique. This test kit operates on the basis of competition between the conjugates and the testosterone in the sample(s) for a limited number of binding sites on the antibody coated wells. Twenty five micro liters of standard or sample(s) was added per micro plate. 200 µl hormone conjugate was added to the standard or sample(s) and covered with a sealing tape. It was incubated at 37 °C for 2 hour. The solution was discarded and microplates washed three times with 300 µl of 1X wash solution. 100 µl of tetramethylbenzidine one step substrate was

added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking. 100 µl of stop solution was added to each microplate. The intensity of the color developed was measured at 450 nm.

Serum progesterone estimation

Serum progesterone was determined by enzyme linked immune sorbent assay technique. This test kit operates on the basis of competition between the conjugates and the testosterone in the sample(s) for a limited number of binding sites on the antibody coated wells. Twenty micro liters of standard or sample(s) was added per micro plate. 200 µl hormone conjugate was added to the standard or sample(s) and covered with a sealing tape. It was incubated at 37 °C for 1 hour. The solution was discarded and microplates washed three times with 300 µl of 1X wash solution. 100 µl of tetramethylbenzidine one step substrate was added to each well and incubated for 15 minutes at room temperature in the dark with gentle shaking.100 µl of stop solution was added to each microplate. The intensity of the color developed was measured at 450 nm.

Statistical analysis

All numerical variables were expressed in mean (± SD) and analyzed using one-way analysis of variance (ANOVA). Using SPSS version 20.0, significant level was considered at $P < 0.05$.

Ethics

Ethical approval was obtained from the ethical committee of the Lahor Research Laboratories and Medical centre in Benin City, Edo State, Nigeria with reference number LRL/010/014.

RESULTS

Our findings revealed a significant decrease in absolute lymphocyte count, $CD4^+$ T cells, progesterone and testosterone concentration of the elderly males when compared with the young males ($P < 0.05$) (Table 1). It also revealed a significant increase in estrogen level of the elderly males when compared with the young males ($P < 0.05$) (Table 1).

DISCUSSION

It is well-established that ageing is associated with a decline in immune function (Gyubeck and Wick, 2002). There is currently paucity published report on the effect of ageing on the immune system and the gonads of male subjects in Benin City, Nigeria, to the best of my knowledge, hence this study. The significant decrease in absolute lymphocyte count and $CD4^+$ T cells count of the elderly as compared with the young males (Table 1).

Table 1: Comparison of mean (±SD) of $CD4^+$ T cell count (cells/µl), estrogen (pg/ml), progesterone (nmol/l), testosterone (ng/ml) and absolute lymphocyte count (cells/µl) of elderly males and young males.

Parameters	CD4+T cells count	Estrogen	Progesterone	Testosterone	Absolute lymphocyte count
Elderly males (n=51)	392±9.76	95± 1.34	2.20± 0.20	3.17± 0.2	1.10± 0.03
Young males (n=51)	810± 17.10	41.1± 1.90	4.37± 1.90	4.12± 0.2	2.74± 0.12
P- value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

P-value < 0.05 was considered significant.

These could be attributed to the shrinkage in the size and function of the thymus gland. This is in accordance with these findings. The shrinking in size and function of the thymus gland may leads to a reduction in the output of new naïve T cells toward the periphery (Hakim et al., 2005). $CD4^+$ T cell pool in young individuals is mainly composed of naïve cells, the proportion of memory cells increases with ageing in both mice and humans (Kovaiou et al., 2005). The major age-associated defect for CD4 T cells has been shown to be a reduction in IL-2 production (Haynes and Lefebvre, 2011). The higher incidence of infectious diseases (influenza), autoimmunity (rheumatoid arthritis), cancer (prostate) and cardiovascular diseases is an obvious issue in ageing (Pawelec, 2007). The most important contributor to the decline in immune function in the elderly is the changes observed in adaptive immunity (Goronzy and Weyand, 2005). Telomeric repeats (TTAGGG) shortening that is associated with ageing has been linked with increased DNA mismatch (Di-Mitri et al., 2011).

A significant decrease in testosterone, progesterone and an increase the level of estrogen in elderly males as compared with the young males ($P< 0.05$) (Table 1). These could be linked to the reduction in the number of Leydig cells of the gonads. Mulligan et al., 2001 opined that the reduction in number of Leydig cells and the increased in the steroidogenic function of the aged gonads could be associated with an increase in the production of estrogen, and a rise in the levels of luteinizing hormone. It is reported that hypogonadism is associated with low testosterone levels, reduced sexual activity, insulin resistance, cognitive decline, cardiovascular events and mortality in ageing males (Yeap, 2009a; Yeap et al., 2012). Decreased testosterone levels are associated with increased risks of osteoporosis, metabolic syndrome and type 2 diabetes mellitus (Yeap, 2009b). Testosterone deficiency should be regarded as a risk factor for cardiovascular disease (Maggio and Basaria, 2009; Jones, 2010).

Conclusion

Ageing induces a decrease in $CD4^+$ T cells count, progesterone and testosterone level. The elevated level of estrogen observed is possibly due to a feedback mechanism associated with hypogonadism. The molecular mechanism needs further investigation.

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