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Studies on some cytokines of apparently healthy Nigerian women aged 10-40 years

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Abstract

The study was done to determine the levels TNF, IL-10, IL-6 and IL-4 in apparently healthy Nigeria women. A total of one hundred and fifty (150) subjects were recruited for the study comprising 50 subjects each for each age group drawn from apparently healthy women from Umuahia. Five milliliters (5ml) of venous blood was collected from each subject and was dispensed into plain containers to obtain serum for the assay of the cytokines. The cytokes were determined by Melsin ELISA kits. The results were expressed as mean± standard deviation. The data were analysed with the statistical package for social science (SPSS) version 21 using t-test, ANOVA and the level of significance was set at P<0.05. The results showed decrease (P<0.05) in IL-10 (20.73±2.05iu/l, 26.13±12.22iu/l, P0.036), when compared among the age groups of the women and no significant difference (P>0.05) in TNF (10.81±0.10iu/l, 10.32±1.21iu/l, P=0.827), IL-4(11.90±8.46iu/l, 44.45±37.95iu/l, P=0.346) and IL-6 (17.73±3.03iu/l, 17.81±2.58iu/l, P=0.543) when compared among the groups. The study showed no significant difference (P>0.05) in TNF (10.81±0.10iu/l, 10.65±1.62iu/l, P=0.862), IL-10 (20.73±2.05iu/l, 26.13±12.22iu/l, P=0.588), IL-4 (11.90±8.46iu/l, 44.45±37.95iu/l, P=0.169) and IL-6 (17.73±3.03iu/l, 17.81±2.58iu/l, P=0.961) when compared between women of 10-20 years and 21-30 years age groups respectively. The results showed no significant difference (P>0.05) in TNF (10.81±0.10iu/l, 10.32±1.21iu/l, P=0.630), IL-10 (20.73±2.05iu/l, 42.54±29.49iu/l, P=0.056), IL-4 (11.90±8.46iu/l, 48.73±15.48iu/l, P=0.165) and IL-6 (17.73±3.03iu/l, 18.97±3.01iu/l, P=0.498) when compared between women of 10-20 years and 31-40 years age groups respectively. The results showed decrease (P<0.05) in IL-10 (26.13±12.22iu/l, 42.54±29.49iu/l, P=0.015) and no significant difference (P>0.05) in TNF (10.65±1.62iu/l, 10.32±1.21iu/l, P=0.576), IL-4 (44.45±37.95iu/l, 48.73±15.48iu/l, P=0.780) and IL-6 (17.81±2.58iu/l, 18.97±3.01iu/l, P=0.279) when compared between women of 10-20 years and 31-40 years age groups respectively. The study showed significant changes among the age groups when interleukin 10 was compared which has multiple effects but plays anti-inflammatory role. Interleukin 10 should be adequately monitored among these women who are at reproductive age to avert immunologically induced diseases.

Keywords: TNF, IL-4, IL-6, IL-10, apparently healthy Nigerian women aged 10-40 years.

Introduction

Tumour Necrotic Factor (TNF) was seen to be formed mainly by macrophages (Walsh *et al.*, 1991), but it is formed also by a many different types of cells involving lymphoid cells, mast cells, endothelial cells, cardiac myocytes, adipose tissue, fibroblasts, and neurons (Swardfager *et al.*, 2010). Huge levels of TNF are made available in connection to lipopolysaccharide, other bacterial products, and Interleukin-1 (IL-1).It is reported that in the skin, mast cells appear to be the major source of pre-formed TNF, which can be available upon inflammatory stimulus (e.g., LPS) (Feng *et al.,* 2015). It has a number of roles on different organ systems, usually linked to IL-1 and Interleukin-6 (IL-6) (Said *et al.,* 2010). A local elevation in level of TNF will lead to cardinal signs of Inflammation to occur. Whereas increased levels of TNF stimulate shock-like symptoms, the prolonged exposure to minimal levels of TNF can lead to cachexia that is a wasting syndrome. This can be seen, for instance, in cancer patients. Said *et al*. showed that TNFα causes an IL-10-dependent inhibition of CD4 T-cell expansion (Starkie *et al.,* 2003).

Studies on cytokines on apparently healthy women in Nigeria are not documented yet and are very important as they are the major immunregulators. Cytokines are low molecular weight regulatory proteins that are secreted by many cells of the immune system in response to a number of stimuli. They are involved in virtually all physiological responses in the body and are key players in coordinating immune responses between cells, by binding to a variety of receptors and to induce cell-specific immune responses. They are secreted by many cells of the immune system in response to a number of stimuli. During successful pregnancies, fetal trophoblasts and maternal leukocytes secrete predominantly T-helper 2 type cytokines to prevent initiation of inflammatory and cytotoxic type responses that might damage the integrity of the materno-fetal placental barrier (Bennett *et al.*, 1999) in response to invading malaria parasites, however it has been documented that Th-1 type cytokines are produce to reverse the Th-2 type bias within the placenta (Rogerson *et al*., 2003). Inconsistence reports on the response of some pro-inflammatory interleukins to peripheral and placental malaria have been documented (Diouf *et al*., 2007; Ismaili *et al*., 2003).

Interleukin 6 (IL-6) acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine (Ferguson-Smith *et al.,* 1998). Interleukin 6 stimulates the inflammatory and auto-immune processes in many diseases such as diabetes (Kristiansen and Mandrup-Poulsen, 2005), atherosclerosis (Dubiński and Zdrojewicz, 2007), depression (Dowlati *et al.,* 2010), Alzheimer's Disease (Swardfager *et al.,* 2010), systemic lupus erythematosus (Tackey *et al.,* 2004), multiple myeloma (Gadó *et al.,* 2000), prostate cancer (Smith *et al.,* 2001), Behçet's disease (Hirohata and Kikuchi, 2012), and rheumatoid arthritis (Nishimoto, 2006).

Interleukin 10 (IL-10) is an anti-inflammatory cytokine (Eskdale *et al.,* 1997). Interleukin 10 is a cytokine with pleiotropic effects in immunoregulation and inflammation. It downregulates the expression of Th1 cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production. IL-10 can block NF-κB activity, and is involved in the regulation of the JAK-STAT signaling pathway. Lower levels of IL-10 have been observed in individuals diagnosed with multiple sclerosis when compared to healthy individuals (Ozenci *et al.,* 1999). Due to a decrease in IL-10 levels, TNFα levels are not regulated effectively as IL-10 regulates the TNF-α-converting enzyme (Brennan *et al.*, 2008). As a result, TNFα levels rise and result in inflammation (Nakahara *et al.,* 2012).

The study was done to determine the levels of cytokines in apparently healthy women aged 10-40 years from Nigeria as they are immunoregulators and affects the haematological parameters and the wellbeing of individuals.

Materials and Methods

Study area

This study was carried out in Umuahia, Abia State.

Subjects

A total of one hundred and fifty (150) subjects were recruited for the study comprising 50 subjects each for each age group drawn from apparently healthy women from Umuahia.

Sample collection

Five milliliters (5ml) of venous blood was collected from each subject and was dispensed into plain containers to obtain serum for the assay of the cytokines.

Laboratory procedures

All reagents were commercially purchased and the manufacturer's standard operating procedures were strictly adhered to.

Alpha tumour necrosis factor (TNF-α) Assay

Human Alpha Tumour Necrosis Factor Commercial ELISA Kit by MELSIN Medical Co Limited was used. Catalogue Number: EKHU-0110

Procedure

Dilutions of standard were prepared to get a concentration of 80 pg/mL, 40png/mL, 20 pg/mL, 10 pg/mL, 5pg/mL and 0pg/mL. 50 uL of standards were pipette into the standard wells. 10uL of test serum were pipette into the each sample well. 40uL of sample dilluent was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, and stop solution). 50 uL of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30minutes at 37^{o}C. It was washed for four times. 50uL of chromogen solution A and 50uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37^{o}C. 50uL of stop solution was added to each well. Optical density of the samples was read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

Interleukin 1 (1L-6) ASSAY

Human Interleukin 6 Commercial ELISA Kit by MELSIN Medical Co Limited was used.
Catalogue Number: EKHU-0102

Procedure

Dilutions of standard was prepared to get a concentration of 240 ng/L, 160 ng/L, 80 ng/L, 40 ng/L, and 20ng/L. 50 uL of standards were pipette into the standard wells. 10uL of test serum were pipette into the each sample well. 40uL of sample dilluent was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, and stop solution). 50 uL of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30minutes at 37^{oC}. It was washed for four times. 50uL of chromogen solution A and 50uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37^{oC}. 50uL of stop solution was added to each well. Optical densities of the samples were read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

Interleukin-10 (1L-10) Assay

Human Interleukin 10 Commercial ELISA Kit by MELSIN Medical Co Limited was used.
Catalogue Number: EKHU-1035

Procedure

Dilutions of standard was prepared to get a concentration of 240 ng/L, 160 ng/L, 80 ng/L, 40 ng/L, and 20ng/L. 50 uL of standards were pipette into the standard wells. 10uL of test serum were pipette into the each sample well. 40uL of sample dilluent was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, and stop solution). 50 uL of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30minutes at 37oC. It was washed for four times. 50uL of chromogen solution A and 50uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37oC. 50uL of stop solution was added to each well. Optical densities of the samples were read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

Interleukin-4 (1L-4) Assay

Human Interleukin 4 Commercial ELISA Kit by MELSIN Medical Co Limited was used.
Catalogue Number: EKHU-0065

Procedure

Dilutions of standard was prepared to get a concentration of 300 ng/L, 200 ng/L, 100 ng/L, 50 ng/L, and 25ng/L. 50 uL of standards were pipette into the standard wells. 10uL of test serum were pipette into the each sample well. 40uL of sample dilluent was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, and stop solution). 50 uL of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30minutes at 37oC. It was washed for four times. 50uL of chromogen solution A and 50uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37oC. 50uL of stop solution was added to each well. Optical densities of the samples were read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

Statistical analysis

The results were expressed as mean± standard deviation. The data were analysed with the statistical package for social science (SPSS) version 21 using t-test, ANOVA and the level of significance was set at $P<0.05$.

Results

Table 1: Mean ±SD values of TNF, IL-10, IL-4 and IL-6 of women of 10-20 years, 21-30 years and 31-40 years age groups

parameters	10-20 years	21-30 years	31-40 years	f-value	p-value
TNF(iu/l)	10.81±0.10	10.65±1.62	10.32±1.21	0.191	0.827^{NS}
IL-10(iu/l)	20.73±2.05	26.13±12.22	42.54±29.49	3.607	0.036^{***}
IL-4(iu/l)	11.90±8.46	44.45±37.95	48.73±15.48	1.090	0.346^{NS}
IL-6(iu/l)	17.73±3.03	17.81±2.58	18.97±3.01	0.620	0.543^{NS}

The results showed decrease (P<0.05) in IL-10 (20.73±2.05iu/l, 26.13±12.22iu/l, P0.036), when compared among the age groups of the women and no significant difference (P>0.05) in TNF (10.81±0.10iu/l, 10.32±1.21iu/l, P=0.827), IL-4(11.90±8.46iu/l, 44.45±37.95iu/l, P=0.346) and IL-6 (17.73±3.03iu/l, 17.81±2.58iu/l, P=0.543) when compared among the groups.

Table 2: **Mean ±SD Values of TNF, IL-10, IL-4 and IL-6 of women of 10-20 years and 21-30 years age groups**

Parameters	10-20 years	21-30 years	p-value
TNF(IU/L)	10.81±0.10	10.65±1.62	0.862^{NS}
IL-10(IU/L)	20.73±2.05	26.13±12.22	0.588^{NS}
IL-4(IU/L)	11.90±8.46	44.45±37.95	0.169^{NS}
IL-6(IU/L)	17.73±3.03	17.81±2.58	0.961^{NS}

The study showed no significant difference (P>0.05) in TNF (10.81±0.10iu/l, 10.65±1.62iu/l, P=0.862), IL-10 (20.73±2.05iu/l, 26.13±12.22iu/l, P=0.588), IL-4 (11.90±8.46iu/l, 44.45±37.95iu/l, P=0.169) and IL-6 (17.73±3.03iu/l, 17.81±2.58iu/l, P=0.961) when compared between women of 10-20 years and 21-30 years age groups respectively.

Table 3: **Mean ±SD values of TNF, IL-10, IL-4 and IL-6 of women of 10-20 years, and 31-40 years age groups**

Parameters	10-20 years	31-40 years	p-value
TNF(iu/l)	10.81±0.10	10.32±1.21	0.630^{NS}
IL-10(iu/l)	20.73±2.05	42.54±29.49	0.056^{NS}
IL-4(iu/l)	11.90±8.46	48.73±15.48	0.165^{NS}
IL-6(iu/l)	17.73±3.03	18.97±3.01	0.498^{NS}

The results showed no significant difference (P>0.05) in TNF (10.81±0.10iu/l, 10.32±1.21iu/l, P=0.630), IL-10 (20.73±2.05iu/l, 42.54±29.49iu/l, P=0.056), IL-4 (11.90±8.46iu/l, 48.73±15.48iu/l, P=0.165) and IL-6 (17.73±3.03iu/l, 18.97±3.01iu/l, P=0.498) when compared between women of 10-20 years and 31-40 years age groups respectively.

Table 4: **Mean ±SD values of TNF, IL-10, IL-4 and IL-6 of women of 21-30 years and 31-40 years age groups**

Parameters	21-30 years	31-40 years	p-value
TNF(iu/l)	10.65±1.62	10.32±1.21	0.576^{NS}
IL-10(iu/l)	26.13±12.22	42.54±29.49	0.015^{*}
IL-4(iu/l)	44.45±37.95	48.73±15.48	0.780^{NS}
IL-6(iu/l)	17.81±2.58	18.97±3.01	0.279^{NS}

The results showed decrease (P<0.05) in IL-10 (26.13±12.22iu/l, 42.54±29.49iu/l, P=0.015) and no significant difference (P>0.05) in TNF (10.65±1.62iu/l, 10.32±1.21iu/l, P=0.576), IL-4 (44.45±37.95iu/l, 48.73±15.48iu/l, P=0.780) and IL-6 (17.81±2.58iu/l, 18.97±3.01iu/l, P=0.279) when compared between women of 10-20 years and 31-40 years age groups respectively.

Discussion

The study showed decrease in IL-10, when compared among the age groups of the women and no significant difference in TNF, IL-4 and IL-6 when compared among the groups. This shows that that age range affects the levels of IL-10 in healthy Nigerian Women. Interleukin 10 may be involved in anti-inflammatory process which is vital to control inflammatory cytokines like the IL-4 and IL-6. The study showed no significant difference in TNF, IL-10, IL-4 and IL-6 when compared between women of 10-20 years and 21-30 years age groups respectively. The results showed no significant difference in TNF, IL-10, IL-4 and IL-6 when compared between women of 10-20 years and 31-40 years age groups respectively. The results showed decrease in IL-10 and no significant difference in TNF, IL-4 and IL-6 when compared between women of 10-20 years and 31-40 years age groups respectively. The clinicians and gynaecologists should monitor IL-10 levels in women which may be a guide in anti-inflammatory process in women in this part of the world. A local elevation in level of TNF will lead to cardinal signs of Inflammation to occur. Whereas increased levels of TNF stimulate shock-like symptoms, the prolonged exposure to minimal levels of TNF can lead to cachexia that is a wasting syndrome. This can be seen, for instance, in cancer patients. Said *et al*. showed that TNFα causes an IL-10-dependent inhibition of CD4 T-cell expansion (Starkie *et al.,* 2003). This study will help in predicting chances of some diseases in women within this area and in preventing the haematological downregulations through these cytokines levels. A balance should be maintained between the inflammatory and anti-inflammatory cytokines to ensure robost immunity and haematopoiesis of the blood cells.

Conclusion

The study showed significant changes among the age groups when interleukin 10 was compared which has multiple effects but plays anti-inflammatory role. Interleukin 10 should be adequately monitored among these women who are at reproductive age to avert immunologically induced diseases. This can be a guide to those involved with management of women's health.

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