Cluster of Differentiation 4, Serum Malondialdehyde and Immunoglobulin M Concentrations in Ageing of Apparently Healthy Humans in Keffi, Nigeria

*Nweze C.C., Solomon M. and Nweze O. A.

1Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria
2Department of Biochemistry, University of Jos, Nigeria
3Department of Medicine and Surgery, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Awka, Nigeria

*Corresponding Email: chibuzoihe@gmail.com

Manuscript received: 24/06/2018     Accepted: 29/06/2018      Published: December 2018

ABSTRACT
This study evaluated blood samples of 150 adults of age range 30-79 years with 96 males and 54 females on immunology indices; malondialdehyde (MDA), immunoglobin M (IgM) and cluster of differentiation 4 (CD\(^+\)). Serum MDA concentration significantly (p<0.05) increased with age. Serum MDA concentration of the males were significantly higher than the females. It indicates that the healthy ageing adult’s antioxidant/oxidant balance is compromised. Thus, the generation of reactive oxygen species (ROS) and ROS clearance has been disturbed and may result in oxidative damage to macromolecules in the cells. In all ages and gender, IgM antibodies to H. pylori was significantly (p<0.05) different and it increased with age. Study showed that IgM antibodies to H. Pylori was significantly (p<0.05) higher in males than females, thus higher level in elderly indicates an increase in autoimmune disease activity. The study showed that there is significant (p<0.05) decrease in CD\(^+\) cells count with age. Males CD\(^+\) cell count were significantly (p<0.05) lower than females, the study showed that CD\(^+\) cell count decreases with each decade in age which shows a reduction in cellular immunity level. The increase in serum MDA and IgM concentration with age, and decrease in CD\(^+\) cell count may affect the immune cells and may increase. Persistent low-grade systemic inflammation that may lead to common pathological processes and its risk factor-metabolic syndrome that are contributing factors to cardiovascular diseases, diabetes, cancer, and many other health risk that may have accounted for different types of immune defects in elderly.

Keywords: Cellular immunity, malondialdehyde, cluster of differentiation 4, Immunoglobulin M
INTRODUCTION

The immune system is a system of biological structures and processes within an organism that protects against diseases. To function properly, an immune system must detect a wide variety of agents, from viruses to parasitic worms, and distinguish them from the organism’s own healthy tissue (Greg and Habicht, 1996). Cellular immune functions and health generally can be compromised by severe nutritional deficiency (Funfe, 2002). They also decline with age, and this decrease might be due to, at least in part, to alterations in nutritional status. The immune cell functions are strongly influenced by the antioxidant/oxidant balance and, therefore, the anti-oxidant levels in these cells play a pivotal role in maintaining immune cells in a reduced environment and in protecting them from oxidative stress and preserving their adequate function (Knight, 1998). More specifically, antioxidants maintain the integrity and function of membrane lipids, cellular proteins, and nucleic acids and the control of signal transduction of gene expression in immune cells. For this reason the immune cells are particularly sensitive to changes in their antioxidant status. Moreover, since the immune system cells have a high percentage of polyunsaturated fatty acids (PUFA) in their plasma membrane. It is not surprising that these cells usually contain higher concentrations of antioxidants than do other cells (Knight, 1998). The immune system is a two-edged sword: the extremely potent and toxic biological effector mechanisms of the immune system can destroy not only threatening microorganisms but also body tissues. Usually the tissue destruction and inflammation associated with the eradication of a microbiological threat are acceptable and functionally insignificant. However, in several human diseases, the immunologically associated tissue destruction and inflammation are harmful, for example, tuberculosis, fulminate hepatitis and meningitis, and, although this may be advantageous to the species as a whole, the effect on the individual may be devastating. It is because of their potential to destroy tissues that the effectors mechanisms of the immune system are very tightly regulated. Innate cells are also important mediators in the activation of adaptive immune system (Bruce et al., 2002). Failure of these regulatory mechanisms results in the full might of the immune system being inappropriately directed against body tissues and the development of autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus (SLE), myasthenia gravis and multiple sclerosis (Devereux et al., 2002). Persistent low-grade systemic inflammation has been increasingly recognized as a common pathological process, and an important contributing factor to cardiovascular diseases and its risk factor—metabolic syndrome. MDA, CD4+ cell counts, and IgM alongside other parameters, are of central importance in the monitoring of immune function (Litman and Dishaw, 2005). During ageing, the balance between the generation of reactive oxygen species (ROS) and ROS clearance can be disturbed resulting in oxidative damage to macromolecules such as membrane phospholipids (Ryter et al., 1985). Malondialdehyde (MDA) is a secondary product of lipid peroxidation and is used as an indicator of tissue damage. The plasma level of MDA is a reliable and common biomarker of the overall lipid peroxidation. Report demonstrated by Nelson and colleagues showed that increased plasma MDA levels in ageing is not only consistent with the role of oxidative stress in ageing, but also supports the idea that plasma MDA levels may be used as a marker of oxidative stress on immunological studies (Nielson et al., 1997).

Cytotoxic cluster of differentiation antigen 4 (CD4+) are types of white blood cells that fight infection it is also called T-helper cells they are made in the spleen, lymph nodes, and thymus gland, which are path of the lymph or infection fighting system. Its measure in the blood entails the capacity of the immune system. These cells control the immune response by directing other cells to perform their respective tasks (McHeyzer-Williams et al., 2006). Immunoglobulin M (IgM) is the first antibody to be produced during an immune response after an initial antigen encounter, as well as the predominant isotype secreted in T-cell independent immune response (Ehrenstein and Notley, 2010). IgM concentration is reactive to wide variety of auto-antigen, and its levels are found markedly elevated in a series of autoimmune diseases (Duarte-Rey et al., 2012). It is therefore believed to be an important component of autoimmunity (Duarte-Rey et al. 2012; Marchalonis et al., 1993). IgM are related to elevated trig, chol, LDL, CD4+, MDA, Uric acid level and white blood cell count. This study is focused on the evaluation of blood biochemical analysis of humans on immunology indices (Immunoglobulin D (IgD), T-lymphocytic count/ Cytotoxic T-cells (CD4+), investigating immune status of both male and female with age variance.

MATERIALS AND METHODS

The study was conducted in Nasarawa State University, Keffi. Keffi is located in Keffi Local Government Area of Nasarawa State, Nigeria. Nasarawa Stateis located in the north central geopolitical zone of
The total volume of reaction mixture

\[ V = \text{volume of sample} \]

F = dilution factor (optional)

E = molar extinction coefficient and

\[ \text{AB} = \text{mean absorbance reading} \]

Titers of antibodies IgM in sera were quantified by evaluating serum antibodies to Helicobacter Pylori (Isotypes of immunoglobulin M (IgM)) and were measured by enzyme immunosorbent assay (ELISA) described by Wernette et al., (2003). The standard and samples duplicate reading were averaged, then the average zero standard optical density were subtracted. A standard curve was prepared by plotting the mean optical density (OD) value for each standard on the y-axis against the concentration on the x-axis and a best fit curve was drawn through the plotted points on the graph.

Cytotoxic cluster differentiation 4 in whole blood was determined using BD FABScount automated machines and reagent kits based on method described by Schmidt (1989). Whole blood sample was added to the reagents, fluorochrome labeled antibodies in the reagents bind specifically to lymphocytes surface reagents. A fixative reagent was added and the sample runs on the instrument. The cells came in contact with laser light which caused it to fluoresce; this provided the information for enumerating the cells. The software identified the T-lymphocyte population and calculated the absolute counts of T-lymphocytes.

The results were analyzed by Pair-wise comparison of the mean validated using analyzeit for Microsoft excel version 10, where a p-value <0.05 was considered statistically significant. Further Post hoc test like the Fisher’s least significant difference (LCD) was used together in the analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The immune related parameters: serum malondialdehyde concentration, serum immunoglobulin concentration and cluster of differentiation 4 cell counts were studied. Serum MDA concentration showed that there was a significance (p<0.05) different in every decade increase in ages of both male and female (table 2). This study agrees with the previous work carried out by Coudray et al., (1997), on the increase of plasma thiobarbituric acid reacting substance, with age, indicating increase on lipid peroxidation. The result shows that males have higher MDA concentration with statistical significant (p<0.05) difference than the female (table 2). This result indicates that MDA level increases with age in a

Nigeria. It lies between latitude 8°35′N and longitude 08°36′E. An overall sample size of 149 was computed for the study. Considering population of area of study at 5% confidence limit, the minimum sample size of 149 was rounded to 150. The study population was determined using CDC EPINFO 7 software statistical package. The study investigated 150 adult volunteers of 96 males and 54 females within age range of 30-79 years old. An inclusion and exclusion criteria was adopted in the study. All reagents and chemicals used were of analytical grade. The volunteers were within the range of body mass index (BMI) of 18 to 36.00 kgm\(^{-2}\) (Table 1).

The scope, nature aim and objectives of the study were thoroughly explained to the volunteers for their consent. All volunteers were made to sign an informed consent letter and questionnaire. The volunteers filled a biodata form that indicated their age and gender and common menu in family diet. Their weight was determined and used to calculate their body mass index. The protocol was reviewed and approved by an ethical committee. An inclusion and exclusion criteria was adopted in selecting the volunteers. The volunteers were grouped according to age ranges of particular gender (30-39, 40-49, 50-59, 60-69, and 70-79 years age ranges of males and females).

Venous blood samples were collected after a 12-14 hour fast by local physicians from the University staff clinic with vacutainer needles and appendof tubes into lithium heparinized vacutainer tubes and sterile bottles for analysis. Serum sample was prepared by centrifugation of the blood samples for 15 minutes at 1000×g. The samples were stored in a refrigerator.

Blood biochemical parameters were analysed/assayed. MDA concentration which is a secondary product of Lipid peroxidation level in serum was estimated spectrophotometrically using Thiobarbituric acid-reactive substances (TBARS) method as described by Walls et al., (1976), and expressed in terms of malondialdehyde (MDA) per µmolof protein. TBA-reactive substances formed in serum sample after a calibrated sample pretreatment procedure primarily consist of MDA which formed pink 1:2 adduct molecules of TBA (MDA-TBA2). The sample was quantified spectrophotometrically from its visible absorbance at 532nm. The Malondialdehyde concentration of the sample was calculated using an extinction coefficient of 1.56×10\(^5\)M-1cm-1 from the equation:

Lipid peroxidation = \( \text{AB}/E \times V/v \times F \), where

\[ V = \text{total volume of reaction mixture} \]
healthy ageing adult Prasher et al., 1992, Cohen et al., 1994. Previous research showed that increased plasma MDA levels in ageing is not only consistent with the role of oxidative stress in ageing but also supports the idea that plasma MDA levels may be used as a marker of oxidative stress on immunological studies Nielsen et al., 1997.

Titters of antibody of helicobacter pylori {Isotype of Immunoglobin M (IgM)} were evaluated. The study showed that there was significant increase in IgM antibody to H. pylori with increase in age (Table 3). The males had significant increase to H. pylori than females (Table 3). Higher IgM antibodies to H. Pylori, indicating the females having lower IgM levels than Males (Gonzalez-Quintela et al., 2008). According to the biochemical indicators, the elderly had higher antibodies when compared to the younger age. IgM concentration is reactive to wide variety of autoantigen, and their levels are found markedly elevated in series of autoimmune diseases (Duarte-Ray et al., 2012). Thus, the elderly are prone to infection and immune disease than the younger.

Cytotoxic cluster of differentiation antigen 4 (CD+4) cell count in the blood, demonstrates the efficacy of the immune system. The study showed that there was a significant (p<0.05) decrease in CD+4 cell count with increase in age both gender (Table 4). Males had lower level of CD+4 cell count than the females with a significance (p<0.05) difference, Females had higher CD+4 cell count than the males (Table 4). CD+4 cell count in both gender decreases with each decade increase in age. Previous studies support the result of the study in Ethiopia, Uganda and India reported a higher CD+4 cell count in females than in males (Tumwebaze, 2012; Uppal et al., 2003; Lee et al, 1996). By contrast, various studies reported a higher CD+4 cell count in males than females, with exception of pregnant women, indicating significant relationship between age and sex to CD+4 cell count (Olumiyiwa et al., 2005; Menard et al, 2003). It is not clear whether there are true variations across countries, in the relationship between gender and CD+4 cell count, or these results are due to confounding factors.

Men and women at advancing age exhibit reduced abilities to mount appropriate antibody responses especially toward new antigens (Jasuja et al., 2013). So far, sex-specific differences in the aging immune system and the effect of declining estrogen and progesterone levels on immunosenescence are poorly understood. At menopause, estradiol production in the ovaries ceases(Nunn et al., 2009). Thereafter, only basal levels of progesterone are being synthetized by the adrenal glands. In aged women, dehydroepiandrosterone (DHEA) and testosterone levels decrease, yet follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels rise from the 4th decade onwards (Al-Azzawi and Palacios, 2009). In men, there is a slower yet steady decline in testosterone levels from their 2nd to 8th decade of life displaying no clear turning point (Bhasin et al., 2011). In turn, estradiol, estrone, LH, and FSH gradually increase (Morley et al., 1997; Jasuja et al., 2013).

The reason for gender differences in immunosenescence is a matter for speculation. There are known to be gender differences in the immune system of males and females. In males the total lymphocyte count is similar to that in females but the percentage of T cells within the lymphocyte population is lower (Bouman et al., 2004; Giltay et al., 2000). There are differences in the function of the immune system in males and females (McCombe et al., 2009; Nunn et al., 2009), and this is probably contributes to the different ability of males and females to deal with infections, and the different prevalence of autoimmune disease in males and females (McCombe et al., 2009). Generally, females produce more vigorous humoral and cellular immune responses than males (Ansar et al., 1985; Weinstein et al., 1984). Some of this may be due to the effects of hormones. For example, estrogen stimulates c-myc which stimulates telomerase, which could have an anti-aging effect (Kyo et al., 1999). Another recent theory relates to the possibility that the evolutionary needs of females and males are different and that mitochondria are better adapted to females than males cells (Tower, 2006).

CONCLUSION
The study showed that with increase in age that the immune system is compromised. Many different types of immune defects in elderly have been identified (Duarte-Ray et al., 2012). Immune status level evaluated and assayed in the study showed that MDA, and IgM increased with age, while CD+4 cell count reduced with age. Many different types of immune defects in elderly have been identified. The previous focus on defects in cell-mediated immune responses provided a possible explanation for the increased risk of cancer, viral infections, and infections with intracellular bacterial pathogens, such as Helicobacter pylori, Mycobacterium tuberculosis, also to pronounced susceptibility to extracellular, bacterial infections, such Streptococcus pneumonia and T-cell deficit infections, all these infections increase with age(Miller, 1996; Haney, 1996).
Table 1: The mean body mass index (BMI) distribution among males and females in different groups

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Age range</th>
<th>BMI (kg/m²)</th>
<th>WHO classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Group 1</td>
<td>30-39</td>
<td>23.00±0.05</td>
<td>21.00±0.05</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>28.00±0.03</td>
<td>26.00±0.04</td>
</tr>
<tr>
<td></td>
<td>50-59</td>
<td>30.00±0.02</td>
<td>28.00±0.05</td>
</tr>
<tr>
<td></td>
<td>60-69</td>
<td>33.00±0.03</td>
<td>30.00±0.02</td>
</tr>
<tr>
<td></td>
<td>70-79</td>
<td>30.00±0.01</td>
<td>33.00±0.01</td>
</tr>
</tbody>
</table>

World Health Organisation, (1989) BMI: <18 = underweight; 18-24.9 = normal/healthy; 25-29.9 = overweight; 30-34.9 = Grade 1 obesity; 35-39.9 = Grade 2 obesity; >40 = Morbid obesity.

Table 2: Serum malondialdehyde concentration (µmol/L)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No of volunteers</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>m=22; f=131.</td>
<td>08±0.08</td>
<td>.43±0.08a</td>
</tr>
<tr>
<td>40-49</td>
<td>m=21; f=13</td>
<td>1.73±0.09b</td>
<td>0.99±0.09a,b</td>
</tr>
<tr>
<td>50-59</td>
<td>m=20; f=11</td>
<td>2.46±0.04b</td>
<td>1.50±0.05a,b</td>
</tr>
<tr>
<td>60-69</td>
<td>m=18; f=9</td>
<td>3.12±0.04b</td>
<td>2.02±0.04a,b</td>
</tr>
<tr>
<td>70-79</td>
<td>m=15; f=8</td>
<td>3.58±0.02b</td>
<td>2.59±0.02a,b</td>
</tr>
</tbody>
</table>

M: males, f: females; a: statistically significant (p<0.05) when compared with serum MDA concentration of corresponding age in male, b: statistically significant when compared with immediate age range in ascending order.

Table 3: Serum immunoglobulin concentration (mg/DL)

<table>
<thead>
<tr>
<th>Ages (years)</th>
<th>No of volunteers</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>m=22; f=13</td>
<td>270±0.08</td>
<td>220±0.07a</td>
</tr>
<tr>
<td>40-49</td>
<td>m=21; f=13</td>
<td>290±0.07b</td>
<td>237±0.07a,b</td>
</tr>
<tr>
<td>50-59</td>
<td>m=20; f=11</td>
<td>343±0.04b</td>
<td>259±0.03a,b</td>
</tr>
<tr>
<td>60-69</td>
<td>m=18; f=9</td>
<td>371±0.03b</td>
<td>268±0.02a,b</td>
</tr>
<tr>
<td>70-79</td>
<td>m=15; f=8</td>
<td>396±0.03b</td>
<td>294±0.02a,b</td>
</tr>
</tbody>
</table>

M: males, f: females; a: statistically significant (p<0.05) when compared with serum IgM concentration of corresponding age in male, b: statistically significant when compared with immediate age range in ascending order.

Table 4: Cluster of differentiation 4 (CD+4) (umo/L)

<table>
<thead>
<tr>
<th>Ages (years)</th>
<th>No of Volunteers</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>m=22; f=13</td>
<td>833±0.08</td>
<td>944±0.07a</td>
</tr>
<tr>
<td>40-49</td>
<td>m=21; f=13</td>
<td>851±0.06b</td>
<td>956±0.07a,b</td>
</tr>
<tr>
<td>50-59</td>
<td>m=20; f=11</td>
<td>869±0.05b</td>
<td>972±0.04a,b</td>
</tr>
<tr>
<td>60-69</td>
<td>m=18; f=9</td>
<td>878±0.03b</td>
<td>988±0.04a,b</td>
</tr>
<tr>
<td>70-79</td>
<td>m=15; f=8</td>
<td>886±0.03b</td>
<td>997±0.03a,b</td>
</tr>
</tbody>
</table>

M: males, f: females; a: statistically significant (p<0.05) when compared with serum IgM concentration of corresponding age in male, b: statistically significant when compared with immediate age range in ascending order.

ACKNOWLEDGEMENT

We acknowledge the cooperation of volunteers in the study. This work is sponsored by Tertiary Education Trust Fund (TETFund).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


Devereux, J.J., Vlachonikolis, I.G., and Buckle, P.W. (2002). Epidemiological study to investigate potential interaction between physical and psychosocial factors at work that may increase the risk of symptoms of musculoskeletal disorder of the neck and upper limb.*PUBMED Occupation and Environmental*
Medicine, 599(4): 269-277.


Olumiyiwa, A., Jelpe D., Manhattan C., Patience A., Silas G, Edwina M., Ruth G, Ndam., Pam D., Comfort


