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CLUSTER OF DIFFERENTIATION 4, SERUM MALONDIALDEHYDE AND IMMUNOGLOBULIN M CONCENTRATIONS IN AGEING OF APPARENTLY HEALTHY HUMANS IN KEFFI, NIGERIA

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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^+_4). 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^+_4 cells count with age. Males CD^+_4 cell count were significantly ($p<0.05$) lower than females, the study showed that CD^+_4 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^+_4 cell count may affect the immune cells and may increase. Persistent low-grade systemic inflammation that may lead tocommom 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 (Funte, 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 erythromytosis (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, CD^{+}_{4}-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 (CD^{+}_{4}) 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 capacityof 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, CD^{+}_{4}, 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 (Immunoglobin D (IgM), T-lymphocytic count/ Cytotoxic T-cells (CD^{+}_{4}), 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

Nigeria. It lies between latitude 80°35N 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 30-79years 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 1.56×10^5M-1cm-1 from the equation:

Lipid peroxidation = AB/E ×V/v × F, where

V = total volume of reaction mixture

V = volume of sample

F = dilution factor (optional)

E = molar extinction coefficient and

AB = 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 immunosorbentassay (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 describe by Schmidt (1989). Whole blood sample was added to the reagents, flourochrome 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 comparism of the mean validated using analyzeit for Microsoft excel version 10, where a p-value <0.05 was considered statically 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 malondial dehydeconcentration, 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

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 Nielson *et al.,* 1997.

Titers of antibody of helicobacter pylori {Isotype of Immunoglobin M (IgM)} were evaluated. The study showed that there was significantn ($p<0.05$) 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 lowerIgM 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^+_4cell 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 are 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^+_4cell 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 tuberculosi*s, 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: Themean body mass Index (BMI)distribution among males and females in different groups

Treatment group	Age range	BMI(kg/m2)		WHO classification	
(years)		Male	Female	Male	Female
Group 1	30-39	23.00+0.05	21.00+0.05	normal	normal
	40-49	28.00+0.03	26.00+0.04	overweight	overweight
	50-59	30.00+0.02	28.00+0.05	grade1obesity	overweight
	60-69	33.00+0.03	30.00+0.02	grade1obesity	grade1obesity
	70-79	30.00+0.01	33.00+0.01	grade 1obesity	grade1 obesity

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)

Age (years)	No of volunteers	Male	Female
30-39	m=22;f=131.	08±0.08	.43±0.08a
40-49	m=21;f=13	1.73±0.09b	0.99±0.09a,b
50-59	m=20;f=11	2.46±0.04b	1.50±0.05a,b
60-69	m=18;f=09	3.12±0.04b	2.02±0.04a,b
70-79	m=15;f=08	3.58±0.02b	2.59±0.02a,b

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)

Ages (years)	No of volunteers	Male	Female
30-39	m=22;f=13	270±0.08	220±0.07a
40-49	m=21;f=13	290±0.07b	237±0.07a,b
50-59	m=20;f=11	343±0.04b	259±0.03a,b
60-69	m=18;f=09	371±0.03b	268±0.02a,b
70-79	m=15;f=08	396±0.03b	294±0.02a,b

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)

Ages (years)	No of Volunteers	Male	Female
30-39	m=22;f=13	833±0.08	944±0.07a
40-49	m=21;f=13	851±0.06b	956±0.07a,b
50-59	m=20;f=11	869±0.05b	972±0.04a,b
60-69	m= 18;f=09	878±0.03b	988±0.04a,b
70-79	m=15; f=08	886±0.03b	997±0.03a,b

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

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Al-Azzawi, F. and Palacios, S. (2009). Hormonal changes during menopause. *Maturitas Journal of endocrinology,63:* 135–137.

Ansar, A.S., Penhale, W.J. &Talal, N. (1985).Sex hormones, immune responses, andautoimmune diseases. Mechanisms of sex hormone action. *American Journal of Pathology, 121*: 531-551.

Bhasin, S., Pencina, M., Jasuja, G.K., Travison, T.G., Coviello, A., Orwoll, E. andVasan, R.S. (2011). Reference ranges for testosterone in men generated using liquid chromatography tandem mass spectrometry in a community-based sample of healthy nonobese young men in the Framingham Heart Study and applied to three geographically distinct cohorts. *Journal of Clinical Endocrinology,* 96: 2430–2439.

Bouman, A., Schipper, M., Heineman, M. J. &Faas, M. M. (2004).Gender difference in the non-specific and specific immune response in humans. *American Journal of Reproduction and Immunology, 52:*19-26.

Bruce, A., Alexander, J., Julian, L, Martin, R. and Keith, R. (2002).Molecular Biology of the cells book 4th Eds. Garland Sciences. U.S.A.

Cohen SM, Olin KL, and Feur W.J. (1994).Low glutathione reductase and peroxidase activity in age- related Macular degeneration. Br. *J Ophtamol, 78*:791-4

Coudray G, Roussel, A.M. and Arnaud, J. (1997). Selenium and Antioxidant Vitamin and Lipid peroxidation levels in Preaging French population. *Gropu. Biol. trace Elempes,57*: 183-90.

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.

Duarte-Rey, C., Bogdanus, D.P., Leung, P.S., Anaya, J.M.and Gershwin, M.E. (2012).IgM Predominance in autoimmune diseases: genetic and gender. *Autoimmune Reviews,11(*6-7): A404-412.

Ehrenstein, M.R.andNotley, C.A. (2010). The importance of Natural Igm: Scavenger, protector and Regulator. *Nature Reviews Immunology,10*: 778-786.

Fuente, D.L.M. Effect of antioxidants on immune system, and aging. Eur. J. clin.NutR.2002; 56: 55-58.

Giltay, E. J., Fonk, J. C., von Blomberg, B. M., Drexhage, H. A., Schalkwijk, C. and Gooren, L. J. (2009). In vivo effects of sex steroids on lymphocyte responsiveness and immunoglobulin levels in humans. *Journal of Clinical Endocrinology,85:*1648-1657.

Giorgi, J.V., Cheng, J.C. andMargolic, J.B. (1990).Quality control in the flow cytometric Measurement of T-lymphocyte subsets; Themulticentre AIDS cohort study experiance. *Clinical Immunology and Immunopathology, 55*: 175-186.

Gonzalez-Quintela A, Alende, R.,Gude, F., Campos, J., Rey, J., Meijide, L.M., Fernandez-Merino, C.andVidal, C.(2008). Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities.*Clinical & Experimental Immunology,151*: 42–50.

Gregory, B. and Habicht, G.S. (1996).The Fabulous complex immune systems of humans and other mammals. *Immunity and the Invertebrates Scientific American, 151*.

Haney L.(1996). Homeboys, babies, men in suits: the state and the reproduction of male dominance,*American Sociological Review, 61*(5): 759-778.

Jasuja, G. K., Travison, T. G., Davda, M., Murabito.,J. M., Basaria, S., Zhang, A. and Bhasin, S. (2013). Age trends in estradiol and estrone levels measured using liquid chromatography tandem mass spectrometry in community-dwelling men of the Framingham Heart Study. *Journal of Gerontology,68:* 733–740.

Klassing, K.C. andleshchinsky, T.V. (2001) Interaction between nutrition and immunity.Nutrition and immunology. In: Gershwin ME, GerJman B and CL Keen (Eds). Humana Press. 20: 363-373.

Knight, J. (1998).Free radicals, their history and current status in aging and disease.*Ann Clin Lab Sci.,28*; 331-46.

Kyo, S., Takakura, M., Kanaya, T., Zhuo, W., Fujimoto, K., Nishio, Y. and Inoue, M. (1999).Estrogen activates telomerase. *International Journal of Cancer, 59*:5917-5921.

Lee BW, Yap HK, Chew FT, Quah TC, Prabhakaran K, Chan GS, Wong SC &Seah CC. Age and sex related changes in lymphocyte subpopulations of healthy Asian subjects: from birth to adulthood. *Cytometry 1996,* 26: 8-15.

Litman, W.G. and Dishaw, L.J. (2005).Changing views of evolution of immunity. *Frontiers in Immunology*, 22: 5-7.

Marchalonis, J.J., Schlutter, S.F., Wilson, L., Yocum, D.E. and Boyer, J.T. (1993). Natural human antibodies to synthetic peptide autoantigens: Correlation with age and autoimmune diseases. *Gerontology,* 39: 65-79.

McHeyzer-Williams, L.J., Malherbe, L.P. and McHeyzer- Williams, M.G.(2006). Chexk points on memory B-cells. *Immunol. Rev., 2*(11): 255-268

McCombe, P. A., Greer, J. M. and Mackay, I. R. (2009).Sexual dimorphism in autoimmune disease.*CurrMol Med,* 19: 1058-1079.

Menard, D., Mandeng, M.J., Tothy, M.B., Kelembho, E.K., Gresenguet,G. andTalarmin, A. (2003). Immunohematological reference ranges for adults from Central African Republic. *Clinical and Diagnostic Laboratory Immunology, 10*: 443-445.

Miller, R. A. (1996). The aging immune system: Primer and prospectus. *Science,* 273: 70-74

Morley, J. E., Kaiser, F. E., Perry-Lii, H. M., Patrick, P., Morley, P. M. K., Stauber, P. M. and Garry, P. J. (1997). Longitudinal changes in testosterone, luteinizing hormone, and follicle-stimulating hormone in healthy older men. *American Journal of Metabolism, 46:* 410–413.

Nielsen, F., Mikkelsen, B.and Nielsen, J.B. (1997). Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clinical Chemistry, 43*:1209-1214.

Nunn, C. L., Lindenfors, P., Pursall, E. R. and Rolff, J. (2009).Sexual dimorphism in immune function.*British Journal of Biological Sciences, 364*: 61-69.

Ohkawa, H., Ohishi, N. andYagi, K. (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry, 2*: 351-358.

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

D., Phyllis K.and Alash'le A. (2005). Epidimology and prevention. Institute of Human Virology; *Clinical Vaccine Immunology. 2005;* 12(4): 523-53.

Prasher S, Pandev SS, and A Gupta antioxidant enzymes in RBC as a biological index ofage related macular degeneration. *Acto Ophtamol* 1992; 71:216-18

Ryter A., Kellenberger E., and Maeder, M.,(1985). Laboratory methods in vesicular and vectorial transport. *Journal of Bacteriology,* 162; 960-971

Schmidt, R.E. (1989). Monoclonal antibodies for diagnosis of immunodeficiencies. Blut 59(3): 200-206.

Tower, J. andArbeitman, M. (2009).The genetics of gender and life span. *Journal of Biology, 8*:38-41.

Tumwebaze, E. (2012). The effect of age, gender and location of residence on CD4 counts response to Arv therapy in Patients who attend nyagatare Hospital in Vct service. BSc Dissertation, Ruhengeri Institute of Higher Education, Rwanda.

Uppal, S.S., Verma, S. andDhot, P.S. (2003).Normal values of CD4 and CD8 lymphocyte subsets in healthy Indian adults and the effects of sex, age, ethnicity and smoking. Cytometry, 52B: 32-36.

Walls, W.K., Kumer, K.S. and Rochstein, P. (1976)Effect of antioxidants on oxidative stressed serum malondialdehyde in-vitro. Journal of I*mmunology, 81:* 227-236.

Weinstein, Y., Ran, S. and Segal, S. (1984): Sex-associated differences in the regulation of immune responses controlled by the MHC of the mouse. *Journal of Immunology,*132: 656-661.Wernette, C.M., Frasch,

C.E., Madore, D., Carlone, G., Goldblatt, D., Plikaytis, B., Benjamin, W., Quataert, S.A., Hildreth, S., Sikkema, D.J., Käyhty, H., Jonsdottir, I. andNahm, M.H, (2003). Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. Clinical and Diagnostic Laboratory Immunology, 10(4): 514-519.