



## THE INFLUENCE OF TWELVE MONTHS NUTRACEUTICALS AND FUNCTIONAL FOODS INTERVENTION ON AGE AND SEX AS RISK FACTORS FOR IMMUNE SYSTEM

<sup>1</sup>Nweze C.C. and <sup>2</sup>Solomon M.

<sup>1</sup>Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria

<sup>2</sup>Department of Biochemistry, Faculty of Health Sciences, University Jos Nigeria

Date Manuscript Received: 12/12/2015 Accepted: 11/05/2016 Published: March 2017

### ABSTRACT

Effect of antioxidant functional foods and nutraceuticals on malondialdehyde (MDA) level, cytotoxic clusters of differentiation antigen 4 (CD4) and Immunoglobulin M (IgM) in male and female individuals were studied for twelve months. The volunteers included a total of 150 healthy adults of 96 men and 54 women aged between 30 and 74 years. They were made to sign an informed consent form, questioner and an ethical approval was gotten. At the baseline, eligible candidates were randomized to either 1 capsule per day of antioxidant nutraceutical (Forever living product) (containing vitamin E 10 mg, vitamin C 60mg and  $\beta$ - carotene 2000 mcg of vitamin A, or cheap affordable indigenous antioxidant functional foods of equivalent vitamin composition of oranges, carrots, and soybean or bottled water, and the first dose was dispensed and followed up for six months. There was a positive decrease in MDA and IgM values and a positive increase in CD4 counts after antioxidant dietary intervention. However, a lower concentration of MDA, and higher level of IgM and CD4 in men than in female at some age ranges was observed. The study showed that the indigenous antioxidant functional foods could be used as an alternative to the expensive imported antioxidant nutraceuticals in boosting immunity and therefore suggest such for immune- compromised individuals especially in developing countries like Nigeria.

**Keywords:** Nutraceuticals, functional foods, antioxidant, malondialdehyde, immunoglobulin M, CD4

## INTRODUCTION

Cellular immune functions and health generally can be compromised by severe nutritional deficiency (De La Fuenete, 2002). They also decline with age, and this decrease might be due to, at least in part, to alterations in nutritional status (Klasing&Leshchinsky, 2001). With increasing knowledge about micronutrients including minerals, vitamins and other compounds like carotenoids, flavonoids, anthocyanins on a molecular level together with results from epidemiological studies open a new and exciting field of nutritional science. Free radicals are incriminated in the pathogenesis of tissue injury in many diseases.

They produce cellular injury by lipid peroxidation, enzyme activation, damage DNA and degradation of structural proteins. The body has evolve multiple defence mechanism through antioxidants against free radicals, these antioxidant include vitamin C, Vitamin E, vitamin A, carotenoids, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), however under normal circumstances, there is a balance between pro-oxidant and antioxidants (Khanna *et al.* 2012). The compound that have been studied most extremely are the antioxidants, many potential benefits have been attributed to antioxidant in the form of antioxidant supplements. These supplements have the potential to alleviate the health problems with ageing caused by excessive production of reactive oxygen species (ROS) (Packer & Werber, 2001). New concepts have appeared with this trend, as nutraceuticals, functional foods, nutritional therapy, phytonutrients, and phytotherapy (Bland, 1996; Berger & Shenkin, 2006; Bagchi, 2006).

The immunesystem is a system of biological structures and processes within an organism that protects against disease (Kahnamooi, Saber&Anzabi, 2013).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, nucleic acids and the control of signal transduction of gene expression in immune cells (Meydani *et al.*, 1995). 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, fulminant 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 effector mechanisms of the immune system are very tightly regulated. Failure of these regulatory mechanisms results in the full might of the immune system being inappropriately directed against body tissues and the development of diseases like rheumatoid arthritis and many other autoimmune diseases.

A food can be regarded as functional if it satisfactorily demonstrated to affect beneficially one or more target functions in the body exceeding just aadequate nutritional effect, in a way which is necessary to either the well being and health or reduction of the risk of infectionsand diseases (Nagpal *et al.*, 2014). Whole foods represent the simplest example of functional foods (carrots, oranges, soybean, avocado pears, and tomatoes) because of their high contents of physiologically active components ( $\beta$ -carotene, vitamin C, vitamin E, and lycopene respectively). “Nutraceutical” is a term coined in 1989 by Stephen DeFelice as food or parts of food that provide medical or health benefits (such as diseases prevention and treatment) (Alissa & Ferns, 2012). Nutraceutical is any nontoxic food extract supplement that has scientifically proven health benefit for both the treatment and prevention of disease (De Felice, 2002).

Free radicals damage contribute to the aetiology of many chronic health problems associated with ageing such as cardiovascular diseases, inflammatory diseases, cataract, cancer, and diabetes (Fang, 2002).Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals and scavenging them (Young & Woodside, 2001). Synthetic antioxidants are recently reported to be dangerous to human health (Balsano&Alisi, 2009). Thus, the search for effective, nontoxic, natural products with antioxidant activity has been intensified

in recent years. In addition to endogenous antioxidant defence systems, consumption of dietary and plant-derived, widely spread, affordable, antioxidants appears to be a suitable alternative. The Nigerian easily grown, widely spread, effective, nontoxic, cheap functional foods (carrots, tomatoes, oranges, soybean and groundnuts) are rich sources of natural antioxidants that should be studied.

There is little evidence of the efficacy of a long term intervention of antioxidant nutraceuticals and functional foods on immune functions in healthy individuals. However, limited or no data is available in support of comparative effects of antioxidant nutraceuticals and functional foods on Malondialdehyde (MDA) level, Cytotoxic cluster of differentiation antigen 4 (CD4) and Immunoglobulin M (IgM) in these subjects according to age and sex distribution. Hence, the present study was undertaken to evaluate the effect of antioxidant nutraceuticals and functional foods on MDA, CD4 and (IgM) levels in healthy subjects according to age and sex distribution.

## MATERIALS AND METHODS

The study was conducted in Nasarawa State University, Keffi, Nigeria. Nasarawa State is located in the north central geopolitical zone of Nigeria. It lies between latitude 80°35'N and longitude 08°36'E. It is bounded to the North-west by Federal capital territory (FCT), Abuja, and to the North-east by plateau state, to the South-east by Taraba state and to the North by Kaduna state, to the South by Benue state and to the South-west by Kogi state (figure I). It has a land mass of 21,117 square kilometre with a population of 2, 100, 000 making it the 10th largest state in Nigeria (Nigerian 2006 census).

The volunteers were randomly assigned to three groups with 3 sub-groups based on three intervals of baseline, six months and twelve months. Group 1; No antioxidant treatment was given for the twelve months but a 75 cl bottled water- placebo group ( Grp 1 (BT)<sub>0m</sub>: placebo baseline group, Grp 1 (AT-P)<sub>6m</sub>: placebo six months group, GRP 1 (AT-P)<sub>12m</sub>: placebo twelve months group). Group 2; the group that later received antioxidant nutraceuticals Forever living product 1 capsule per day of (vitamin E (10mg), vitamin C (60mg) and  $\beta$ - carotene(2,000mcg of vit A)-nutraceutical treatment group (Grp 2 (AT-N)<sub>0m</sub>: baseline group that were later given antioxidant nutraceuticals, Grp 2 (AT.N)<sub>6m</sub>: nutraceutical treatment group that received antioxidant for six months, Grp 2 (AT-N)<sub>12m</sub>: nutraceutical treatment group that received antioxidant for twelve months). Group 3; the group that later received antioxidant

functional foods of equivalent vitamin composition oranges (100g), carrots (100g), 75cl soybean drink and a heaped tablespoon of powdered soybean (35g) daily-functional group treatment group (Grp 3 (AT-F)<sub>0m</sub>: baseline group that was later given antioxidant, Grp 3 (AT-F)<sub>6m</sub>: functional food treatment group that received antioxidant for six months, Grp 3 (AT-F)<sub>12m</sub>: functional food treatment group that received antioxidant for twelve months).

The study subjects included a total of 150 healthy adults of 96 men and 54 women aged between 30 and 74 years. All volunteers are staff of Nasarawa State University, Keffi. A Single-centred open dose study was undertaken by the 150 healthy subjects (96 men and 54 women. The inclusion and exclusion criteria were that the subjects had no history of gastrointestinal surgery, or other significant pathology, were non-smokers, had no history of alcohol or drug abuse, were non-diabetic, were not on a calorie-reduced or vegetable diet nor were taking antioxidant/vitamin supplement, female were not pregnant or lactating. No concomitant medication was allowed throughout the study except contraceptive pill.

The scope, nature, aim and objectives of this study were thoroughly explained to voluntary participants for their consent, and all of them were made to sign an informed consent letter and a questionnaire.

Three hundred (300) volunteers' venous blood samples were taken by local physicians. Volunteers with desirable health status were chosen. Most of the people not chosen have either HIV+ve, hepatitis B or C, very high/low blood sugar ( $\geq 7.77$  and  $\leq 3.33$  mmol/L), extremely low/high blood pressure ( $< 100/60$  mmHg and  $> 140/95$  mmHg), those that are not sure of their date of birth. Some due to personal reasons refuse to participate. At the end we had 180 assumed healthy participants. Volunteers who were assumed healthy were randomly assigned to three groups as in dietary intervention above. At six months of dietary intervention blood samples were collected from all groups for biochemical analysis just as at baseline.

Lipid peroxidation (Malondialdehyde, MDA) level in serum were estimated spectrophotometrically using Thiobarbituric acid- reactive substances (TBARS) method as described by Ohkawa, Ohishi & Yagi, 1979). Cytotoxic cluster of differentiation antigen 4 (CD4) in blood serum was determined using BD Facscount machines using the method described by Giorgi, Cheng & Margolic (1990). Immunoglobulin M (IgM antibody) Titers of antibodies IgM in sera were quantified by evaluating serum antibodies to

*Helicobacter Pylori* (Isotypes of immunoglobulin M(IgM)) were measured by enzyme immunoassay (EIA) using goat-antihuman IgM with alkaline phosphatase (EC 3.1.3.1) (Wernette *et al.* 2003).

The experimental results were expressed as the mean  $\pm$  SD. The parameters were analysed statistically using one-way analysis of variance (ANOVA) for comparing the mean using Smith's Statistical package (SSP).

## RESULTS AND DISCUSSION

Immune status results after six months intervention of antioxidants nutraceuticals and antioxidant functional foods are shown in table 1-3. The result in table 1 showed that males have higher MDA concentration than females even after antioxidants treatment. The result showed that the MDA of antioxidant treated groups were positively influenced than the placebo group at six and twelve months (Grp 1 (AT-P)<sub>6m</sub> and 12m) and the indigenous antioxidant functional foods (Grp 3 (AT-F)<sub>6m and 12m</sub>) were affected more positively with decrease in MDA than the imported nutraceutical antioxidant (Grp 2 (AT-N)<sub>6m and 12m</sub>). The result showed that male MDA levels were mostly higher than the female of same age range. Result showed that the MDA levels of males and females decrease with increase in age. Statistically, the Serum MDA level of Grp 1 (AT-P)<sub>6m and 12m</sub> showed statistically difference with negative increase in MDA except for the males of age range of 30-39 years and males of age range 40-49 compare to Grp 1 (BT)<sub>0m</sub>. Grp 2 (AT-N)<sub>6m and 12m</sub> showed a significant difference in all ages of male and female except for males in 40-49 age range compared to Grp 2 (BT)<sub>0m</sub>. Grp 3 (AT-F)<sub>6m and 12m</sub> showed a significant different in positive decrease of all age groups for male and females compared to Grp 3 (BT)<sub>0m</sub>.

The result in Table 2 showed that the serum CD4 count of antioxidant treated groups were positively influenced than the placebo group ( Grp1 (AT-P)<sub>6m and 12m</sub>). Grp 3 (AT-F)<sub>6m and 12m</sub> were affected more positively than the antioxidant Grp 2 (AT-N)<sub>6m and 12m</sub>. There was a statistical difference in all the treatment groups at six and twelve. There is a difference in serum CD4 level of different ages of male and female after treatment. Result showed that the serum CD4 levels of males and females decrease with increase in age. The result showed that most of the female has good increase reference values of CD4 than the male before and after treatment. The result showed that most of the placebo groups did not show any improvement on any range that was not within the reference range like in ages 60-69 of the placebo group the values did not improve within the six and twelve months rather

it negatively reduced ( Grp 1 (BT)<sub>0m</sub>=55, Grp 1 (AT-P)<sub>6m</sub>=50). The older adult from 60-79 have serum IgM concentration that is either above or below the reference range though some of the antioxidant group were slightly improved at that age. The IgM values are more positive in the younger adults than older adults. Generally, from the result more of the males have better serum IgM concentration than the females. Statistically, there was a significant difference between Grp 2 (AT-N)<sub>12m</sub> and Grp 2 (AT-N)<sub>6m</sub> and Grp 2 (BT)<sub>0m</sub> except for the females in age groups 70-79. Also there is a significant difference between Grp 3 (AT-F)<sub>12m</sub>, Grp 3 (AT-F)<sub>6m</sub> and Grp 3 (BT)<sub>0m</sub> though some of them are within normal reference ranges.

This randomized, prospective, parallel group, comparative, open dose and single centre study was conducted to determine the effects of antioxidant nutraceuticals and functional foods intervention on Malondialdehyde (MDA), Cytotoxic cluster of differentiation antigen 4 (CD4) and Immunoglobulin M (IgM) in relation to age and sex distribution as risk factors for immuno-compromised individuals. Immune cells are influenced by antioxidant/oxidant balance; the antioxidant levels play a vital role in maintaining immune cells in a reduced environment and in protecting them from oxidative stress (Knight, 1998). Because the immune system is critically dependent on accurate cell-cell communication in order to mount a response, immune cell integrity is essential. The effects of antioxidants are very beneficial during period of oxidative stress like the periods of infections, and in elderly. In the study the blood parameters evaluated and assayed relates to their levels in the blood with ageing and most times sex. The antioxidant intervention positively influenced the blood parameters with the antioxidant functional foods showing a better influence than the antioxidant nutraceuticals.

Persistent low-grade systemic inflammation has been increasingly recognized as a common tissue destruction process, and an important contributing factor to cardiovascular diseases and its risk factor-metabolic syndrome. MDA, CD4+-cell counts, and IgM is one of central important parameter in monitoring of immune function (Tumwebaze,2012). 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.

In the study, the immune status level evaluated and assayed are in table 1 it shows that MDA increases with age, while CD4 count and IgM reduces with age. The result showed that the younger males have

lower malondialdehyde than younger females; the males have good increase reference values of CD4 and Immunoglobulin M than the female. Figure 1 to 3 showed a significant improvement on the immune status level with antioxidant dietary intervention with the antioxidant functional foods given better results than the antioxidant nutraceuticals. 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 pronounced susceptibility to extracellular bacterial infections, such as *Streptococcus pneumoniae* and T-cell deficit infections, all these infections increase with age (Miller, 1996). Malondialdehyde (MDA) is a secondary product of lipid peroxidation and is used as an indicator of tissue damage. MDA results agreed with a previous work that plasma thiobarbituric acid reacting substances increases with age, indicating increases lipid peroxidation (Coudray *et al.* 1997). Previous studies showed that patients with immune diseases have higher increase in MDA with increase in ageing than healthy ageing adult group (Prasher, Pandev & Gupta, 1993; Cohen, Olin & Feur, 1994). The raw result also showed that males have higher MDA concentration than females. The plasma level of MDA is a reliable and common biomarker of the overall lipid peroxidation. Nielsen, Mikkelsen & Nielsen (1997) 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.

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 lymphoid organs (spleen, lymph nodes and thymus gland) which are part of the lymph or infection fighting system. Its measure in the blood shows how strong the immune system is. CD4 results showed that there is a significant decrease in CD4 count with age. The results showed that males have good increase reference value of CD4 count than the females. A study support the result of these study, age and sex is significantly related to CD4 count, men have lower CD4 count than women, and CD4 count decreases with each decade increase in age, exclusion of pregnant women in a HIV +ve study (Aina *et al.* 2005). A study in Ethiopia disagrees with this result that women have higher CD4 count than men (Lee *et al.*, 1996) but studies among Indians (Uppal,

Verma & Dhot, 2003) and also in Uganda (Tugume *et al.* 1995) agrees with this result. It is not clear whether there are true variations across countries in the relationship between gender and CD4 cell counts or these results are due to confounding factors. Our result showed that there is significant increase in CD4 cell counts on age of the elderly. This is consistent with previously reported findings in Central African republic (Menard *et al.* 2003) it reported significant increase in CD4 cell count by decades in age.

After an initial antigen encounter, Immunoglobulin M (IgM) is said to be the first antibody to be produced during an immune response and is the predominant isotype secreted in T-cell independent of immune response (Ehrenstein & Notley, 2010). IgM concentration is reactive to wide variety of autoantigen, 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 *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. Titers of antibody to *Helicobacter pylori* (Isotype of Immunoglobulin M (IgM)) were evaluated, results are shown in table 1. It showed that female has higher antibodies-IgM antibodies to *H. pylori* than male. Our result is consistent with previous studies, that females have higher IgM levels than males (Gonzalez-Quintelan *et al.* 2008) although the precise mechanism is yet unclear, the stimulatory action of estrogens' on B-lymphocytes could be the cause (Bouman, Heineman & Faas, 2005). A large number of studies have established that most autoimmune diseases occurs significantly more frequently in females than males (Gleicher & Barad, 2007). The results showed that there is a significant decrease on IgM antibody to *H. pylori* with increase in age. According to the biochemical indicators, the elderly has lower antibodies when compared to the younger age. Thus, the elderly are more prone to infections and immune diseases than the young. The reference range for IgM is between (55-575 mg/Dl) higher levels of IgM above the upper limit can mean mononucleosis, macroglobulinemia, rheumatoid arthritis, early viral hepatitis kidney damage (nephritic syndrome, or a parasite infection; lower level of IgM below the lower limit occur in multiple myeloma, some types of leukaemia, and in some inherited types of immune diseases (Ehrenstein & Notley, 2010).

## CONCLUSION

In conclusion, data of the present study suggest that the dietary intervention of antioxidant nutraceuticals and functional foods intervention is likely to

compromise immune functions. These findings also showed possible effects of age and gender on immune parameters of healthy individuals treated with antioxidant diets and supplements and this should pave a way for more research on antioxidants functional foods. Findings from this study showing lower levels of MDA and higher level of CD4 and IgM among men, point out to an advantage of males in relation to females. We recommend more intact of antioxidant functional food as an alternate

to antioxidant nutraceuticals for both healthy and immuno-compromised individuals.

**ACKNOWLEDGEMENT**

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

**CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

**Table 1: Effect of Antioxidants on Serum Malondialdehyde Level (µmol/L)**

Groups	30-39		40-49		50-59		60-69		70-79		
	M	F	M	F	M	F	M	F	M	F	
Baseline											
Grp 1 (BT) <sub>0m</sub>	0.88±0.04	0.87±0.02	0.93±0.09	0.89±0.10	1.36±0.08	1.48±0.09	1.52±0.07	1.58±0.06	2.00±0.04	1.91±0.00	
Grp 2 (BT) <sub>0</sub>	0.91±0.03	0.88±0.03	0.96±0.09	0.90±0.11	1.22±0.08	1.32±0.08	1.92±0.06	1.81±0.05	2.30±0.00	2.18±0.04	
Grp 3 (BT) <sub>0m</sub>	0.87±0.03	0.90±0.04	0.98±0.09	0.93±0.08	1.25±0.10	1.27±0.11	2.00±0.06	1.98±0.07	2.29±0.04	2.16±0.00	
Antioxidant Treatment (6 months)											
Grp1AT-P) <sub>6m</sub>	0.89±0.04	0.85±0.03a	0.93±0.09	0.87±0.08a	1.38±0.07a	1.50±0.06a	1.60±0.06a	1.60±0.02a	2.20±0.02a	2.00±0.00a	
Grp2AT-N) <sub>6</sub>	0.95±0.02b	1.00±0.03b	0.95±0.05	1.10±0.04b	1.20±0.04b	1.20±0.06b	1.75±0.03b	1.50±0.03b	1.90±0.00b	1.88±0.01b	
Grp3AT-F) <sub>6m</sub>	0.90±0.03c*	1.10±0.04c	1.00±0.05c*	1.12±0.05c*	1.20±0.07 c	1.15±0.06c*	1.70±0.03c*	1.35±0.02c*	1.85±0.01c*	1.70±0.00c*	
Antioxidant Treatment (12 months)											
Grp1AT-P) <sub>12m</sub>	0.90±0.04d	0.86±0.03d	0.95±0.09 d	0.90±0.08d	1.52±0.07 d	1.50±0.07	1.65±0.06d	1.60±0.03	2.25±0.03d	2.00±0.00	
Grp2AT-N) <sub>12m</sub>	0.90±0.03e	0.89±0.03e	0.92±0.06 e	1.08±0.08e	1.15±0.04e	1.18±0.07e	1.45±0.04e	1.40±0.04 e	1.65±0.00e	1.62±0.3e	
Grp3AT-F) <sub>12m</sub>	0.89±0.03f	0.88±0.04 f	0.92±0.08 f	0.91±0.07f	1.13±0.08f	1.10±0.08 f	1.40±0.05f	1.30±0.05f	1.50±0.02f	1.45±0.00f	

Reference value: 0.88-1.61 (µmol/L); Grp 1 (BT): Group 1 before treatment; Grp 2 (BT): Group two before treatment; Grp 3 (BT): Group 3 before treatment; Grp 1 (AT-P)<sub>6m</sub>: Group 1 after treatment- placebo; Grp 2 (AT-N)<sub>6m</sub>: Group 2 after treatment- nutraceuticals; Grp 3 (AT-F)<sub>6m</sub>: Group 3 after treatment- functional food; Grp 1 (AT-P)<sub>12m</sub>: Group 1 after twelve months of no treatment; Grp 2 (AT-N)<sub>12m</sub>: Group 2 after twelve months treatment-nutraceuticals; Grp 3 (AT-F)<sub>12m</sub>: Group 3 after twelve months treatment-functional foods; p<0.05 was considered statistically significant; n: 150; a: was considered statistically significant when compared with Grp 1 (BT); b: was considered statistically significant when compared with Grp 2 (BT); c: was considered statistically significant when compared with Grp 3 (BT); \*: was considered statistically significant when compared with Grp 2 (AT-F)<sub>6m</sub>; d: was considered statistically significant when compared with Grp 1 (AT-P)<sub>6m</sub>; e: was considered statistically significant when compared with Grp 2 (AT-N)<sub>6m</sub>; f: was considered statistically significant when compared with Grp 3 (AT-F)<sub>6m</sub>.

**Table 2: Effect of Antioxidants on Serum Cytotoxic Clusters Differentiation Antigen 4 Level (µmol/L)**

Groups	30-39		40-49		50-59		60-69		70-79		
	M	F	M	F	M	F	M	F	M	F	
Baseline											
Grp 1 (BT) <sub>0m</sub>	944.02±0.03	981.01±0.01	935.04±0.07	1028.08±0.12	898.05±0.09	500.05±0.08	680.03±0.04	510.03±0.04	512.01±0.02	550.60±0.00	
Grp 2 (BT) <sub>0m</sub>	810.01±0.02	1004.02±0.03	813.06±0.10	780.05±0.09	463.07±0.11	773.07±0.11	705.03±0.05	801.03±0.05	432.25±0.00	569.03±0.04	
Grp 3 (BT) <sub>0m</sub>	999.01±0.02	992.02±0.03	960.08±0.12	817.07±0.11	708.05±0.09	482.04±0.07	501.03±0.05	760.03±0.05	476.01±0.01	522.76±0.00	
Antioxidant Treatment (6 months)											
Grp 1 (AT-P) <sub>6m</sub>	925.03±0.04a	985.01±0.02a	920.02±0.03a	1030.05±0.08a	900.04±0.07a	510.04±0.06a	600.03±0.04a	555.01±0.02a	500.01±0.02a	600.50±0.00a	
Grp 2 (AT-N) <sub>6m</sub>	840.02±0.03b	1010.02±0.03b	845.06±0.10b	800.05±0.09b	500.05±0.08b	850.05±0.08b	730.02±0.03b	900.02±0.03b	490.45±0.00b	600.01±0.01b	
Grp 3 (AT-F) <sub>6m</sub>	1010.01±0.02c*	1000.03±0.04c*	990.06±0.10c*	850.05±0.08c*	740.05±0.08c*	580.04±0.05c*	540.03±0.04c*	800.02±0.03c*	510.01±0.01c*	600.45±0.00c	
Antioxidant Treatment (12 months)											
Grp 1 (AT-P) <sub>12m</sub>	925.02±0.03	987.01±0.01d	920.03±0.05	955.06±0.10d	550.05±0.08d	900.04±0.07d	550.03±0.04d	600.02±0.03d	500.01±0.02	580.60±0.00d	
Grp 2 (AT-N) <sub>12m</sub>	880.02±0.03e	950.02±0.03e	850.07±0.11e	900.05±0.08e	550.06±0.10e	855.05±0.09e	750.03±0.04e	810.03±0.04e	610.38±0.00e	600.01±0.02e	
Grp 3 (AT-F) <sub>12m</sub>	950.02±0.03f	1000.02±0.03	940.07±0.11f	950.05±0.09f	750.05±0.08f	800.04±0.06f	600.04±0.05f	800.03±0.04	550.01±0.01f	650.65±0.00f	

Reference value: 500-1000(µmol/L); Grp 1 (BT): Group 1 before treatment; Grp 2 (BT): Group two before treatment; Grp 3 (BT): Group 3 before treatment; Grp 1 (AT-P)<sub>6m</sub>: Group 1 after treatment- placebo; Grp 2 (AT-N)<sub>6m</sub>: Group 2 after treatment- nutraceuticals; Grp 3 (AT-F)<sub>6m</sub>: Group 3 after treatment- functional food; Grp 1 (AT-P)<sub>12m</sub>: Group 1 after twelve months of no treatment; Grp 2 (AT-N)<sub>12m</sub>: Group 2 after twelve months treatment-nutraceuticals; Grp 3 (AT-F)<sub>12m</sub>: Group 3 after twelve months treatment-functional foods; p<0.05 was considered statistically significant; n: 150; a: was considered statistically significant when compared with Grp 1 (BT); b: was considered statistically significant when compared with Grp 2 (BT); c: was considered statistically significant when compared with Grp 3 (BT); \*: was considered statistically significant when compared with Grp 2 (AT-F)<sub>6m</sub>; d: was considered statistically significant when compared with Grp 1 (AT-P)<sub>6m</sub>; e: was considered statistically significant when compared with Grp 2 (AT-N)<sub>6m</sub>; f: was considered statistically significant when compared with Grp 3 (AT-F)<sub>6m</sub>.

**Table 3: Effect of Antioxidants on Serum Immunoglobulin M Level (mg/dL)**

Groups	30-39		40-49		50-59		60-69		70-79	
	M	F	M	F	M	F	M	F	M	F
Baseline(0m)										
Grp 1 (BT) <sub>0m</sub>	270.03±0.04	188.01±0.01	304.04±0.07	249.08±0.12	140.05±0.09	225.06±0.10	52.04±0.06	55.05±0.08	375.01±0.03	377.70±0.00
Grp 2 (BT) <sub>0m</sub>	306.02±0.03	205.03±0.04	122.02±0.03	162.03±0.04	211.05±0.9	231.06±0.10	53.06±0.10	378.05±0.09	50.05±0.06	381.01±0.02
Grp 3 (BT) <sub>0m</sub>	259.01±0.02	303.02±0.03	371.05±0.09	367.06±0.10	107.07±0.11	167.04±0.07	54.05±0.08	54.04±0.06	49.03±0.04	52.45±0.00
Antioxidant Treatment (6 months)										
Grp 1 (AT-P) <sub>6m</sub>	270.03±0.04	180.01±0.02a	300.04±0.07a	240.05±0.09a	145.03±0.05a	225.04±0.06	50.04 ± 0.06a	50.02 ± 0.03a	378.02±0.03a	380.50±0.00a
Grp 2 (AT-N) <sub>6m</sub>	316.02±0.03b	220.01±0.02b	132.04±0.07b	180.03±0.04b	220.06±0.10b	240.04±0.06b	64.01 ± 0.02b	370.01±0.02b	60.30 ± 0.00b	380.01±0.01
Grp 3 (AT-F) <sub>6m</sub>	270.01±0.02c*	300.02±0.03c*	360.04±0.07c*	355.03±0.04c*	120.05±0.09c*	170.02±0.03c*	68.04 ± 0.06c*	60.01±0.02c*	59.01 ± 0.02c	54.35±0.00c*
Antioxidant Treatment (12 months)										
Grp1AT-P <sub>12m</sub>	270.03±0.04	180.01±0.01	315.04±0.06d	245.06±0.10d	150.05±0.08d	250.05±0.08d	50.03±0.05	50.03±0.05	379.01±0.02d	382.34±0.00d
Grp2 AT-N <sub>12m</sub>	300.09±0.13e	200.02±0.03e	130.03±0.05e	120.02±0.03e	240.05±0.08e	220.05±0.09e	80.03±0.05e	350.03±0.04e	5.45±0.00e	370.01±0.01e
Grp3AT-F <sub>12m</sub>	280.02±0.03f	310.02±0.03f	350.04±0.07f	360.04±0.07f	130.06±0.10f	180.03±0.05f	75.03±0.05f	65.02±0.04f	65.02±0.03f	60.38±0.00f

Reference value: 55-375 (mg/dL); Grp 1 (BT): Group 1 before treatment; Grp 2 (BT): Group two before treatment; Grp 3 (BT): Group 3 before treatment; Grp 1 (AT-P)<sub>6m</sub>: Group 1 after treatment- placebo; Grp 2 (AT-N)<sub>6m</sub>: Group 2 after treatment- nutraceuticals; Grp 3 (AT-F)<sub>6m</sub>: Group 3 after treatment- functional food; Grp 1 (AT-P)<sub>12m</sub>: Group 1 after twelve months of no treatment; Grp 2 (AT-N)<sub>12m</sub>: Group 2 after twelve months treatment-nutraceuticals; Grp 3 (AT-F)<sub>12m</sub>: Group 3 after twelve months treatment-functional foods; p<0.05 was considered statistically significant; n: 150; a: was considered statistically significant when compared with Grp 1 (BT); b: was considered statistically significant when compared with Grp 2 (BT); c: was considered statistically significant when compared with Grp 3 (BT); \*: was considered statistically significant when compared with Grp 2 (AT-F)<sub>6m</sub>; d: was considered statistically significant when compared with Grp 1 (AT-P)<sub>6m</sub>; e: was considered statistically significant when compared with Grp 2 (AT-N)<sub>6m</sub>; f: was considered statistically significant when compared with Grp 3 (AT-F)<sub>6m</sub>.

**REFERENCES**

Aina O, Dadik J, Charurat M, Amangaman P, Gurumdi S, Mang E, Guyit R, Lar N, Datong P, Daniyam C, Kanki P & Abimiku A (2005). Reference Values of CD4 T Lymphocytes in Human Immunodeficiency Virus-Negative Adult Nigerians. *Clinical and Diagnostic Laboratory Immunology* 12(4): 525–530.

Alissa EM & Ferns GA (2012). Functional Foods and Nutraceuticals in the Primary Prevention of Cardiovascular Diseases. *Journal of Nutrition and Metabolism*, 569486: 1-16.

Bagchi D (2006). Nutraceuticals and functional foods regulations in the United States and around the world. *Toxicology* 221: 1-3.

Balsano C & Alisi A (2009). Antioxidant effects of natural bioactive compounds. *Current Pharmaceutical Design* 134: 3479S-3485S.

Berger MM & Shenkin A (2006). Vitamins and trace elements: Practical aspects of supplementation. *Nutrition* 22: 952-955.

Bland JS (1996). Phytonutrition, phytotherapy and phytopharmacology. *Alternative Therapies in Health And Medicine* 2: 73-76.

Bouman A, Heineman MJ & Faas MM (2005) Sex hormones and the immune response in humans. *Human Reproduction Update* 11: 411–423.

Cohen SM, Olin KL & Feur WJ (1994). Low glutathione reductase and peroxidase activity in age-related Macular degeneration. *British Journal of Ophthalmology* 78: 791-794

Coudray G, Roussel A.M., Arnaud J & Favier A (1997). Selenium and Antioxidant Vitamin and Lipid peroxidation levels in Preaging French population. EVA Study Group. *Edude de vieillissement artériel. Biological Trace Element Research* 57(2):183-190.

De La Fuente M. (2002). Effect of antioxidants on immune system, and aging. *European Journal of Clinical Nutrition* 56: 55-58.

Duarte-Rey C, Bogdanus DP, Leung PS, Anaya JM & Gershwin ME (2012). IgM Predominance in autoimmune diseases: genetic and gender. *Autoimmune Reviews* 11(6-7): A404-412.

Ehrenstein MR & Notley CA (2010). The importance of Natural Igm: Scavenger, protector and Regulator. *Nature Review of Immunology* 10: 778-786.

Ehrenstein MR & Notley CA (2010). The importance of Natural Igm: Scavenger, protector and Regulator. *Nature Reviews Immunology* 10: 778-786.

Fang ET (2002). Free radicals, antioxidants, and nutrition. *Nutrition* 18: 872-879.

- Giorgi J.V., Cheng JC & Margolic J.B. (1990). Quality control in the flow cytometric Measurement of T-lymphocyte subsets; The multicentre AIDS cohort study experience. *Clinical Immunology and Immunopathology* 55: 175-186.
- Gleicher N & Barad DH (2007). Gender as risk factor for autoimmune diseases. *Journal of Autoimmune* 28: 1-6.
- Gonzalez-Quintela A, Alende R, Gude F, Campos J, Rey J, Meijide LM, Fernandez-Merino C, Vidal 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.
- Kahnamooi HS, Saber APR & Anzabi Y (2013). The Effect of Immunostim on Sheep's Immune System. *Bulletin of Environment, Pharmacology and Life Sciences* 2(12): 21-26.
- Khanna HD, Karki K, Pande D & Negi R (2012). Oxidative stress, antioxidant status, regulation of Gene expression and angiogenesis in carcinogenesis. *Indian Journal of Research* 6: 4-14.
- Klasing KC & Leshchinsky TV (2001). Interaction between nutrition and immunity : Lessons from animal agriculture. Gershwin ME, German B & Keen CL (Eds). *Nutrition and immunology: Principles and practice*. Humana press, NJ. Pp. 363-373.
- Knight J (1998). Free radicals: their history and current status in aging and disease. *Annals of Clinical & Laboratory Science* 28: 331-146.
- Lee BW, Yap HK, Chew FT, Quah TC, Prabhakaran K, Chan GS, Wong SC & Seah CC (1996). Age and sex related changes in lymphocyte subpopulations of healthy Asian subjects: from birth to adulthood. *Cytometry* 26: 8-15.
- Marchalonis JJ, Schlutter SF, Wilson L, Yocum DE & Boyer JT (1993). Natural human antibodies to synthetic peptide autoantigens: Correlation with age and autoimmune diseases. *Gerontology* 39:65-79.
- Menard D., Mandeng M. J., Tothy M. B., Kelembho EK, Gresenguet G & Talarmin A (2003). Immunohematological reference ranges for adults from Central African Republic. *Clinical and Diagnostic Laboratory Immunology* 10: 443-445.
- Meydani SN, Wu D, Santos MS & Hayek MG (1995). Antioxidants and immune response in aged persons: overview of present evidence. *American Journal of Clinical Nutrition* 62: 1462S-1476S.
- Miller R.A. (1996) The aging immune system: Primer and prospectus. *Science* 273: 70-74
- Nagpal R, Yadav H, Kumar M, Jain S, Yamashiro Y & Marotta F (2014). Probiotics, Prebiotics and Synbiotics: An Introduction. In: *Probiotics and Prebiotics in Food, Nutrition and Health*. Otles S. (Ed). Taylor & Francis Group, LLC. UK.
- Nielsen F, Mikkelsen B & Nielsen JB (1997). Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clinical Chemistry* 43:1209-1214.
- Ohkawa H, Ohishi N & Yagi K (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry* 2: 351-358.
- Packer L & Weilber SU (2001). The role of vitamin E in the emerging field of nutraceuticals. Kramer K, Happer PP & Parker L (eds). *Nutraceuticals in health and diseases prevention*. Marcel Dekkar, New York. Pp. 27-43.
- Prasher S., Pandev S.S. & Gupta A., (1993). Antioxidant enzymes in RBC as a biological index of age related macular degeneration. *Acta Ophthalmologica* 71: 216-218
- Schmidt R.E., (1989). Monoclonal antibodies for diagnosis of immunodeficiencies. *Blut* 59(3): 200-206.
- Tugume SB, Piwowar EM, Lutalo T., Mugenyi P.N., Grant R.M., Mangeni F.W., Pattishall K. & Katongole-Mbidde E (1995). Hematological reference ranges among healthy Ugandans. *Clinical and Diagnostic Laboratory Immunology* 2: 233-235.
- 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 SS, Verma S & Dhot PS (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.
- Wernette CM, Frasch CE, Madore D, Carlone G, Goldblatt D, Plikaytis B, Benjamin W, Quataert SA, Hildreth S, Sikkema DJ, Käyhty H, Jonsdottir I & Nahm MH (2003). Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. *Clinical and Diagnostic Laboratory Immunology* 10(4): 514-519.
- Young IS & Woodside JV (2001). Antioxidants in health and disease. *Journal of Clinical Pathology* 54: 176-186.