Available online at www.elixirpublishers.com (Elixir International Journal)

Food Science

Elixir Food Science 88 (2015) 36423-36427

ISSN: 2229-712X

Immune Systems Response to Changes in Antioxidants: The Influence of Six Months Nutraceuticals and Functional Foods Intervention on Age and Sex as Risk Factors for Immuno-Compromised Individuals

Carole Chibuzo Nweze[1,*], Mariam Solomon[2] and Mustapha Abdullahi Alqasim[1]

[1]Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria.

[2]Department of Biochemistry, University of Jos, Nigeria.

ARTICLE INFO

Article history:

Received: 07 June 2015;

Received in revised form:

10 November 2015;

Accepted: 17 November 2015;

Keywords

Antioxidant nutraceuticals,
Antioxidant functional foods,
Cytotoxic cluster of differentiation antigen 4 (CD_4),
Immunoglobulin M (IgM),
Age and sex.

ABSTRACT

To assess the long-term effects of antioxidants in individuals according to age and sex distribution.The studied subjects included a total of 150 healthy adults of 96 men and 54 women aged between 30 and 74 years. At the baseline visit, eligible candidate were randomized to either 1 capsule per day of antioxidant nutraceutical or antioxidant functional foods or placebo, and the first dose was dispensed and followed up for six months.The results were grouped based on gender and age ranges, a positive increase in Immunoglobulin M (IgM) and Cytotoxic cluster of differentiation antigen 4 (CD_4) counts after antioxidant dietary intervention and higher level of IgM and CD4 in men than in female at some age ranges were observed.The authors suggest the use of antioxidant functional foods as an alternative to antioxidant nutraceuticals in boosting immunity and therefore suggest such for immuno-compromised individuals.

© 2015 Elixir All rights reserved.

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; Bagchi, 2006).

The immune system 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 polyunsatured 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 a adequate nutritional effect, in a way which is necessary to either the well-being and health or reduction of the risk of infections and diseases (Nagpal *et al.* 2014). Whole foods represent the simplest example of functional

Tele:
E-mail addresses: chibuzoihe@gmail.com

© 2015 Elixir All rights reserved

foods (carrots, oranges, soybean, avocado pears, and tomatoes) because of their high contents of physiologically active components (β-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 Cytotoxic cluster of differentiation antigen 4 (CD_4) 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 CD_4 and (IgM) levels in healthy subjects according to age and sex distribution.

Material and Methods

Study Area

The study was conducted in Nasarawa State, Nigeria. Nasarawa State is located in the north central geopolitical zone of Nigeria. It lies between latitude $80^0$35'N and longitude $08^0$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. It has a land mass of 21,117 square kilometre with a population of 2, 100, 000 making it the 10^{th} largest state in Nigeria according to Nigerian 2006 population census.

Nutraceutical and Functional Foods Intervention

The volunteers were randomly assigned to three groups. Group 1 (control group) received oral antioxidant nutraceutical (Forever living product) 1 capsule per day (containing vitamin E 10mg, vitamin C 60mg and β- carotene 2,000mcg of vit A). Group 2 (treatment group) received antioxidant functional foods of equivalent vitamin composition oranges, carrots, and soybean).

Study Population

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.

Study Design, Inclusion and Exclusion Criteria

A randomized, prospective, parallel group, comparative, open dose and single centre study was undertaken by the150 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.

Ethical Review and Independent Monitoring

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 questioneer. The protocol was reviewed and approved by the Chairman Ethical committee, Federal Ministry of Health Abuja through the Chief Medical Director, Hospital Management Board Lafia Nasarawa State and the Medical Director, Nasarawa State University Medical Clinic, Keffi. The study was independently monitored by an Ethical committee desk officer from Federal Ministry of Health, Abuja according to Quality Assurance programme such that Good clinical practice were followed throughout the one year study.

Specimen Collection and Laboratory Analysis

Three hundred (300) volunteers' veinous blood samples were taken by local physicians from the university staff clinic in 3rd week of June, 2012 and clinical records were taken. 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$3.33mmol/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.

Six Months Dietary Intervention and Samples Collection and Evaluation

Baseline samples were collected six months ago and the blood parameters analysed as reported in these six months antioxidants dietary intervention. Six months blood samples were drawn from the volunteers who are assumed healthy according to their groups, after a 12-14 hour fast, in a 0.1% EDTA tubes, Lithium hyperinized bottles, and sterile bottles for biochemical analysis. Volunteers were six months ago randomly assigned to groups of three. Control group volunteers were given antioxidant nutraceuticals ((Forever living product) 1 capsule per day of vitamin E (10mg), vitamin C (60mg) and β- carotene (2,000mcg of vitamin A)). The treatment group volunteers were given antioxidant functional foods of equivalent vitamin composition (oranges (100g), carrots (100g), and soybean drink (75cl) and 1 heaped table spoon of soybean powder (35g/day)). The placebo group volunteers were giving clean drinking water (ordinary Swan table water (75cl) with no antioxidants).

Analysis of Immunological Status

Absolute CD4 counts have been used to evaluate the immune status of patients with, or suspected of developing immune deficiencies such AIDS (Schimdt, 1989). 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).

Statistical Analysis

Pair-wise comparisons of the means were validated using Analyze-It for Microsoft Excel Version 10, where a p-value < 0.05 was considered statistically significant. Further Post hoc test like the Fischer's least significant difference (LSD) was used together in the analysis of variance (ANOVA).

Post hoc test (LSD) are designed for situations in which researcher has obtained a significant omnibus F-test with a factor that consists of three or more means and additional exploration of the differences among means so as to provide specific information on which means are significantly different from each other.

Result

Immune status results after six months intervention of antioxidants nutraceuticals and antioxidant functional foods are shown in table 1. Cytotoxic cluster of differentiation antigen 4 (CD_4) showed mainly a positive decrease in most of the cases after antioxidant intervention, while those that are above the reference range showed positive decrease to the normal range (See Table 1). CD_4 in blood serum showed that there was a significant decrease the sex with LSD-Male (p-value) 203.48 (0.0033) and female 228.00 (0.0147). Hence, no significant decreased was observed in age with LSD-Gender (p-value) 392.91 (0.5536). The result also showed that the younger males of age range 30-39 (Male = 1010 ± 0.02), 40-49 (Male = 990 ± 0.10) and 50-59 (Males = 740 ± 0.08) has good increase reference values of CD4 than in females (Female = 1000 ± 0.04), (Female = 850 ± 0.08) and (Female = 580 ± 0.05) of the same age ranges after treatment respectively. However, lower concentration of CD4 in older males was seen at 60-69 (Males = 540 ± 0.04) and 70-79 (Males = 510 ± 0.01) than in females (Female = 800 ± 0.03) and (Female = 600±0.00) of the same age ranges respectively.

Immunoglobulin M (IgM) in serum showed a positive decrease or increase to the reference range after antioxidant intervention (Table 1). However, IgM in blood serum showed no significant difference in age and sexes with LSD-Gender (p-value) 197.54 (0.2173), LSD-Male (p-value) 184.25 (0.1205) and LSD-Female (p-value) 186.66 (0.3840). Moreover, the results of Immunoglobulin M (IgM) in males after treatment showed that their concentrations in the blood are lower at age range of 30-39 (Male = 270.00±0.02) and 50-59 (Male = 120.00±0.09) than in females (Female = 300.00±0.03) and (Female = 170.00±0.03) of the same age ranges respectively. Higher Immunoglobulin M (IgM) was seen higher in males at age range of 40-49 in males (Males = 360.00±0.07), 60-69 (Males = 68.00 ± 0.06) and 70-79 (Males = 59.00 ± 0.02) than in females (Female = 355.00±0.04), (Female = 60.00±0.02) and (Female = 54.00±0.00) of the same age ranges respectively.

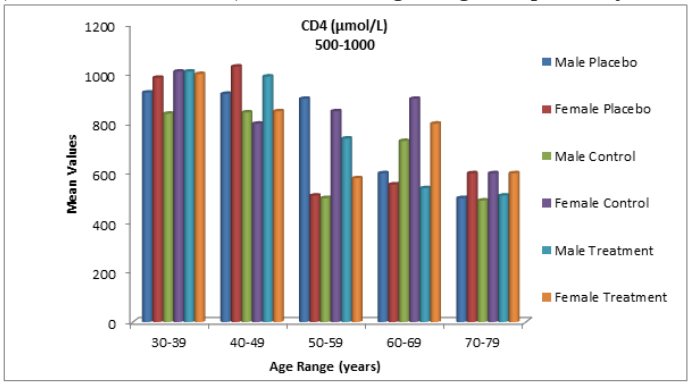

Figure 1. Effect of antioxidants on CD4 after six months treatment

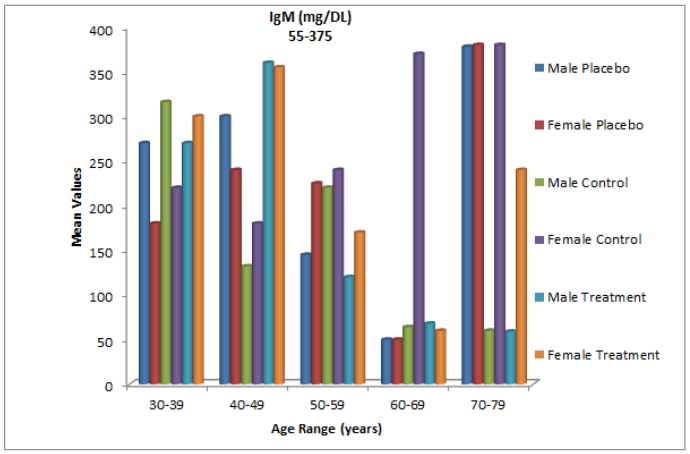

Figure 2. Effect of antioxidants on IgM after six months treatment

Discussion

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 Cytotoxic cluster of differentiation antigen 4 (CD_4) 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. CD_{4+}-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 CD_4 count and IgM reduces with age. The result showed that the younger males have good increase reference values of CD_4 and Immunoglobulin M than the female. Figure 1 and 2 showed a significant improvement on the immune status level with antioxidant dietary intervention with the antioxidant functional foods given better results than the antioxidant nutrceuticals. 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 pneumonia and T-cell deficit infections, all these infections increase with age (Miller, 1996).

Table 1.Effect of antioxidant nutraceuticals and functional foods on Immune Status after six months treatment

		CD4 (µmol/L)	IgM (mg/DL)
Group	**Age(years)**	**Male**	**Male**
PLACEBO (n=31)	30-39(n=08)	925±0.04	270.00±0.04
	40-49(n=09)	920±0.03	300.00±0.07
	50-59(n=06)	900±0.07	145.00±0.05
	60-69(n=05)	600±0.04	50.00 ± 0.06
	70-79(n=03)	500±0.02	378.00±0.03
CONTROL (n=32)	30-39(n=10)	840±0.03	316.00±0.03
	40-49(n=10)	845±0.10	132.00±0.07
	50-59(n=06)	500±0.08	220.00±0.10
	60-69(n=05)	730±0.03	64.00 ± 0.02
	70-79(n=01)	490±0.00	60.00 ± 0.00
TREATMENT (n=31)	30-39(n=09)	1010±0.02	270.00±0.02
	40-49(n=10)	990±0.10	360.00±0.07
	50-59(n=07)	740±0.08	120.00±0.09
	60-69(n=03)	540±0.04	68.00 ± 0.06
	70-79(n=02)	510±0.01	59.00 ± 0.02
		Female	Female
PLACEBO (n=19)	30-39(n=07)	985±0.02	180.00±0.02
	40-49(n=06)	1030±0.08	240.00±0.09
	50-59(n=03)	510±0.06	225.00±0.06
	60-69(n=02)	555±0.02	50.00 ± 0.03
	70-79(n=01)	600±0.00	380.00±0.00
CONTROL (n=18)	30-39(n=05)	1010±0.03	220.00±0.02
	40-49(n=05)	800±0.09	180.00±0.04
	50-59(n=04)	850±0.08	240.00±0.06
	60-69(n=02)	900±0.03	370.00±0.02
	70-79(n=02)	600±0.01	380.00±0.01
TREATMENT (n=19)	30-39(n=06)	1000±0.04	300.00±0.03
	40-49(n=06)	850±0.08	355.00±0.04
	50-59(n=04)	580±0.05	170.00±0.03
	60-69(n=02)	800±0.03	60.00±0.02
	70-79(n=01)	600±0.00	54.00±0.00
LSD- Gender (p-value)		392.91 (0.5536*)	197.54 (0.2173*)
LSD-Male (p-value)		203.48 (0.0033)	184.25 (0.1205*)
LSD-Female (p-value)		228.00 (0.0147)	86.66 (0.3840*)

* Not Significant; Normal/reference range: CD4: 500-1500µmol/L and IgM: 55-375mg/dL

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

Conflict of Interest

The authors declare no conflict of interest.

Reference

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.

Bland JS (1996). Phytonutrition, pyhtotherapy 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.

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.

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

Giorgi JV, Cheng JC & Margolic JB (1990). Quality control in the flow cytometric Measurement of T-lymphocyte subsets; The multicentre AIDS cohort study experiance. *Clinical Immunology and Immunopathology* 55: 175-186.

Gleicher N & Barad DH (2007). Gender as risk fact 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 immunolology: 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.

Menard D, Mandeng MJ, Tothy MB, 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.

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.

Schmidt RE (1989). Monoclonal antibodies for diagnosis of immunodeficiencies. *Blut* 59(3): 200-206.

Tugume SB, Piwowar EM, Lutalo T, Mugyenyi PN, Grant RM, Mangeni FW, 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.