

Original Article

Relationship between stress levels and the status of serum antioxidant vitamins

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ABSTRACT

Background: Alternative methods for stress monitoring and evaluation prove very useful in proper dealing with it. Thus, establishing a link between stress exposure and serum antioxidant vitamins' levels would certainly provide a clue towards stress management. **Aim:** The study aimed at evaluating the status of serum antioxidant vitamins in stress conditions. **Methods:** The study was carried out on a total of fifteen Bayero University Kano students of comparable age. Data on their general lifestyle, nutritional habit and study/reading pattern, their blood samples were collected. Serum levels of vitamins A and vitamin C were also assayed. **Result:** The serum vitamins' mean values were compared before, during and after the commencement of examination for statistical significant difference. When compared on before versus after commencement of examination, mean serum vitamin A levels was found to be significantly lower before than after the commencement of examination while no significant difference existed between the corresponding serum vitamin C levels. Moreover when compared during versus after and before versus during the commencement of examination, both the mean serum vitamins' levels were found to be significantly lower during than before and after the commencement of examination. **Conclusion:** According Findings from this study showed that serum vitamins A and C levels declined most during the commencement of examination. This might be an indicator of highest stress level attained by the study subjects at the period. This study confirms that the higher the stress level, the lower the status of serum antioxidant vitamins.

Key Words: Stress, oxidative stress, antioxidant, vitamin A, Vitamin C

INTRODUCTION

Stress can be described as environmental changes that must be adapted to if health

and life are to be maintained; an event that elicits increased cortisol and adrenaline secretions.^[1] During every moment of our waking lives we are conscious of experience



of one kind or the other. These experiences may be stressful or unstressful, depending upon many factors, including genetic predisposition, former psychological conditioning, physical health and mental attitudes.^[2] Cognitive and emotional signs and symptoms include memory problems, inability to concentrate, poor judgement, anxious and racing thought, constant worrying, inability to relax, moodiness, sense of loneliness and isolation and depression of general happiness.^[2] The stress response is the body's way of protection. It helps one stay focused and alert, giving one an extra stress for defense when faced with stressful situation. However, beyond a certain point stress stops being helpful and starts causing major damages to health, mood, productivity and the overall quality of life.^[1]

Long-term exposure to stress can lead to serious health problems, chronic stress can disrupt vital systems in the body with serious effects ranging from hypertension, heart attack and stroke to suppressing immune system, enhancement of aging process due to consequent occurrence of oxidative stress and degenerative diseases.^[1] Wu, 2004 found that endurance exercise and stress can increase oxygen utilization from 10 to 20 times over the resting state and that greatly increases the generation of free radicals, prompting concern about enhanced damage to muscles and other tissues.^[3] When there is imbalance between the generation of these free radicals and the ability of body to neutralize them, then oxidative stress is said to occur. Reactive oxygen species are also used in cell signalling, a dubbed redox signalling.^[4]

The term antioxidant originally was used to refer specifically to a chemical that prevents the consumption of oxygen.^[5] Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats which is the cause of rancidity.^[6] The possible mechanisms of action of antioxidant were first explored when it was recognized that a substance with oxidant activity is likely to be one that is itself readily oxidized.^[7] The reactive oxygen species produced in the cell include hydrogen peroxides (H_2O_2), hypochlorous acid (HOCl) and free radicals such as hydroxyl radicals ($\cdot OH$) and superoxide

anion ($\cdot O_2$).^[7] The hydroxyl radical is particularly unstable and reacts rapidly and non-specifically with most biological molecules.^[8,9] These oxidants can damage cells, starting from chain reaction such as lipid peroxidation or by oxidizing DNA or protein.^[9] Damages to DNA cause mutations and possible cancers if not repaired by DNA repair mechanism,^[10] while damage to protein causes enzyme inhibition, denaturation and protein degradation.^[11]

Enzymatic and non-enzymatic mechanisms are involved in antioxidant defense. Common antioxidants includes the vitamins A, C and E, glutathione and the enzymes superoxides dismutase, catalase, glutathione peroxidase and glutathione reductase amongst others. Vitamin C and E inhibit hydroperoxide formation, metal complexing agents such as penicillamine and bind transition metals involved in some reactions, such as, Fenton and Haber-Weiss-type reactions.^[12] According to the result of the study by Bjelakovic and his team in 2007 at Copenhagen University Hospital beta carotene, vitamins A and E usage were associated with increased mortality.^[13] In the study 68 different trial encompassing 232,606 in total were sorted into high-bias risk and low-bias risk based on the quality of the method employed.^[13]

Most of the studies cited above and many other related ones paid more attention to the effects of the oxidative stress or the use of antioxidants than establishing a comprehensive relationship between the potential source of the oxidative stress (emotional stress), the oxidative stress itself and most important antioxidants in the process.^[4,10] Vitamin C is known to play a significant role in dealing with oxidants by sparing the functions of both vitamin A and E due to its outstanding property of reversible oxidation between ascorbic acid and dehydroascorbic acid and.^[14] This property also enables the molecule to play a significant role in stress response. Henceforth establishing a logical relationship between these serum vitamins' status under a variety of stress exposure conditions is of paramount importance, which is the aim of this study.

METHODOLOGY

Study site and subjects, sample collection and preparation

The study was carried out at Bayero University Kano, Department of Biochemistry and Molecular Biology. A total of fifteen Bayero university Kano students (8 males and 7 female, ages between 22-30 years) served as volunteering study subjects. They were selected from different departments and faculties randomly with no inclusion and exclusion criteria because students mainly, tend to adjust their study style/ reading habit as exam approaches or during exam. Thus, any undergraduate students could serve as subjects for the study. These subjects were maintained throughout the study. Information on their study styles, nutritional habits and general life style was recorded by means of questionnaire prior to sample collection. Venous blood (5ml) was collected from each subject, centrifuged and the sera stored. These samples were collected two weeks before the university examination. The same procedure was repeated during and after the commencement of the examination on the same subjects.

Determination of serum vitamin A was carried out as outlined by Pett and Lepage (1940)^[15]

Determination of Serum vitamin C level was determined according to the method of Roy and Kuether (1943).^[16]

The study was approved by the Ethical Committee of the institution, and informed consent was obtained from each subject.

Statistical analysis

The collected raw data was subjected to paired comparison analysis^[17] followed by ANOVA and Student t-test^[17] under 14 degree of freedom and 5% level of significance.

RESULT

The serum vitamins' mean values were compared on before, during and after the commencement of examination to check for statistical significant difference. When compared on before versus after commencement of examination mean serum

vitamin A levels was found to differ significantly ($P < 0.05$), being lower before than after the commencement of examination while no significant difference ($P > 0.05$) between the corresponding serum vitamin C levels. Moreover When compared on during versus after and before versus during the commencement of examination, both the mean serum vitamins' levels were found to be lower (statistically significant) ($P < 0.05$) during than before and after the commencement of examination.

DISCUSSION

Serum antioxidant vitamins (A and C) status before, during and after commencement of examination reflects stress levels of the study subjects. When compared on before versus after commencement of examination mean serum vitamin A levels was found to be significantly lower before than after the commencement of examination while no significant difference between the corresponding serum vitamin C levels differed. This might have been as a result that the study subjects have suffered acute stress at these periods. The acute stress caused the serum vitamin A level to fall down but not enough to cause serum vitamin C level to decline. When compared on both before versus during and during versus after the commencement of examination, both mean serum vitamins' (A and C) levels were significantly lower ($P < 0.05$) during than before and after the commencement of examination.

Moreover, according to sex, mean serum vitamins A and C levels for both male and female subjects was found to decrease significantly across the periods, with their lowest levels during the commencement of examination. In all the periods mean serum vitamins' levels for male subjects was found to be higher than that of female subjects. This observation points to the fact that male subjects tend to develop stress coping ability although the serum vitamins are known to be slightly higher naturally, in male than female.

The fact that vitamin C is demanded for the synthesis of stress hormones by the adrenal gland in the period of stress^[1] and that, it is required for the synthesis and release of histamine during stress situation could justify

this sudden fall in its level. Emotional stress (due to fear anxiety, constant worrying and the change in reading pattern) undergone by the study subject during the commencement of examination might have caused higher demand of vitamin C by the adrenal tissue (tissue concerned with the synthesis and the release of cortisol, hormone for stress respond). As vitamin C is diverted from circulation to adrenal tissue for the synthesis

of cortisol on the course of stress response, its antioxidant capacity is thought to be reduced leading to the insufficient dealing with the free radicals generated during bodily physiologic and biochemical processes.^[12] The balance between their generation and the ability of the antioxidant defense system to neutralize them is disrupted. Thus, oxidative stress is said to have occurred.

Table 1: Serum vitamins A and B levels before, during and after the commencement of examination and BMI of the study subjects irrespective of sex

Period	Vitamin A($\mu\text{g}/\text{dl}$)	Vitamin C (mg/dl)	BMI (kg/M)
Before examination n= 15	59.50 \pm 10.96 ^a	1.58 \pm 0.19 ^b	39.04 \pm 5.92
During examination n= 15	48.10 \pm 10.40	1.39 \pm 0.33 ^b	39.04 \pm 5.92
After examination n= 15	55.61 \pm 12.08 ^a	1.52 \pm 0.28	39.04 \pm 0.28

Values are mean \pm standard deviation, values with similar superscript are significant at $P < 0.05$

Table 2: Serum vitamins A and B levels before, during and after the commencement of examination and BMI of the study subjects according to sex

Period	Vitamin A($\mu\text{g}/\text{dl}$)	Vitamin C (mg/dl)	BMI (kg/M)
Before examination Male (n=8)	62.40 \pm 10.93 ^a	1.56 \pm 0.19 ^c	36.45 \pm 4.02
Female (n=7)	53.64 \pm 4.51 ^b	1.41 \pm 0.27 ^d	35.02 \pm 5.91
During examination Male (n=8)	47.10 \pm 10.40 ^a	1.32 \pm 0.33 ^c	36.45 \pm 4.02
Female(n=7)	46.31 \pm 5.22 ^b	1.26 \pm 0.46 ^d	35.02 \pm 5.91
After examination Male (n=8)	52.61 \pm 9.08 ^a	1.54 \pm 0.28 ^c	36.45 \pm 4.02
Female(n=7)	50.44 \pm 8.34 ^b	1.44 \pm 0.23 ^d	35.02 \pm 5.91

Values are mean \pm standard deviation, values with similar superscript are significant at $P < 0.05$

Beta carotene being the precursor of vitamin A is thought to protect against oxidative stress.^[18] Vitamin A requires the sparing action of vitamin C to stabilize the free radicals, thus, when serum vitamin C is depleted, even if vitamins A or E captures the free radicals, they cannot be reoxidised

back, hence serum vitamin A level is also thought to drop.^[19]

CONCLUSION

According to the result of this research work, serum antioxidant vitamins' (A and C) levels

declined most during the commencement of examination, thus, the study subjects are thought to have undergone the highest level of stress at that period. It follows that, the higher the stress level the lower the serum antioxidant vitamins' (A and C) status. However, the sample size of the study is a limitation. The study experienced difficulty in finding enough volunteers as the study subjects had to be available to donate blood sample at the three consecutive periods, thus leading to the smaller number of samples. The attrition rate was 50%; initially 30 subjects volunteered to participate in the study but only 15 subjects were available in the course of the study. We recommend further research to study the variation of the ability to cope with stress amongst human races at the genetic level.

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