Malondialdehyde (MDA) as biomarker for oxidative stress in university students

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Abstract

Objective:

To assess the association of MDA levels with Stress, Anxiety and depression in university students from Karachi, Pakistan.

Methodology:

This is a comparative, cross-sectional study conducted in national university of science and arts, Karachi. The sample size was 69, modified Satoh method was used to quantify the amounts of MDA, while for psychological assessment, DASS (Depression, Anxiety, and Stress Scale) was used. Statistical package of social sciences (SPSS) version 21 was used to analyze the data.

Results:

Mean age was 18.4 ± 4.8 years, over all MDA mean value was determined as 3.05 ± 0.3. DASS scores were reported as higher in first year students while 16-19 years of age as compared to other groups indicating higher level of psychological issues in newly admitted students.

Conclusion:

According to the foregoing findings, the MDA levels of second-year students are higher than those of first-year & third-year students, indicating a higher level of oxidative stress in second-year students. As a result, additional work on health enhancement can be done in this population.

Keywords:

MDA, Oxidative stress, DASS

Introduction:

With increasing competition in the academic world, more and more students are opting for professional education and are very keen to go to diverse universities to pursue their dreams.

University life as whole, is a new step in life and needs significant changes in routine and demands thorough adaptation. The change in time, study schedule and new peers can collectively cause stress and anxiety among the students. These stress triggers, when unmanageable and persistent, can cause unavoidable oxidative stress in university students.

Several factors lead to the increase in stress in university individuals. Changes in examination pattern with a widespread syllabus also trigger stress and can cause physical and psychosomatic dysfunction in anxious individuals.[1]

Oxidative stress in the long run, effects the molecular biology of a person and ultimately leads to derangement in standard bodily functions owing to the production of free radicals. Free radicals or reactive oxygen species (ROS) including peroxides, hydrogen peroxide, hydroxyl ions, and superoxide anions form within the body but are metabolized with the help of enzymes namely, superoxide dismutase, catalase and glutathione peroxidase and are either excreted or consumed within the body, to protect the cells from their harmful effects. But in case of increased oxidative stress, the burden of free radicals increase and therefore they get accumulated within the normal tissues.[2]

Accumulation of excessive ROS lead to radical chain reaction and then attack the cell membrane phospholipids and can cause membrane disruption by lipid peroxidation, leading to cell damage and death. Also at nuclear level, the free radical chain reactions damage the DNA directly leading to cell death. Lipid peroxidation ends in the formation of a byproduct malondialdehyde (MDA) which is used to quantify oxidative stress by laboratory evaluation.[3,4]

Furthermore, oxidants like vitamins C and E, help the healthy human body by blocking the radical chain reaction, hence proving the role of healthy diet in preventing physiological degeneration.[5]

University students are prone to stress and anxiety. Literature proves a direct association between exam stress and increased production of ROS. Furthermore, the high school and school study hours are lesser than university hours which consume a good part of the day. Also, longer travelling distance of the institute from residence is tiring and exhausting and is a huge stress contributor to the already crammed routine. The dilemma of covering the entire

core curriculum and competition with the colleagues cause further exhaustion and sleep deprivation in trying to cope up with the induced peer pressure.[6]

Individuals with poor coping mechanism or with added responsibilities of the family are can give up very easily and are more prone to the added stress of the exerting routine.

The financial burden when inflicted on the students, especially males, causes an additive stress in the students. Also, the students who work and earn alongside their studies live a very stressful routine.[7]

Lack of family time and lack of leisure in order to catch up with the mounting syllabus also leads to added stress. Poor physical health and inability to incorporate exercise and workout in the routine adds up to the added stress response. Exercise releases endorphins that can suppress the ROS reaction chain and physically make an individual feel better. University routine seldom allows any individual to become the Jack of all trades.[8]

The stress of the future with the constant thoughts of employment options and further studies alongside the already prevalent exhaustion causes a chain of anxiety and distress.

The cumulative effect of aforementioned factors leads to psychological, physiological and metabolic disturbances in the body and can trigger the ROS formation, ultimately ending up in ROS chain reactions.

Oxidative stress as a result starts a vicious cycle of producing ROS which ultimately leads to the cell membrane disruption. MDA levels in the serum are used as an objective parameter to assess the severity of oxidative stress. Also, the levels of superoxide dismutase and catalase can also be measured in the serum to assess and quantify the oxidative stress.[9]

Furthermore, total antioxidant capacity (TAC) is another objective parameter to evaluate the point of discussion.

Studies done also prove that students suffer more oxidative stress measured in form of MDA levels during examination days.

MDA levels are measured by Ferric Reducing Antioxidant Power (FRAP) and Thiobarbituric acid reactive substance (TBARS) assay in the laboratory that can easily assess the numerical levels of MDA.

Stress response also results in increased cortisol production that can start the metabolic syndrome at a young age and further lead to diabetes, hypertension and obesity. All these physiological disturbances are interlinked with each other and can co-exist or lead to one another in the long run.[10]

In our study, we will be discussing several determinants of oxidative stress in university students, different variables impacting it, and the long term effect of this oxidative burden physiological and psychological health.

Methodology:

The current study included biological sciences students from the first and third year students from private university of Karachi to investigate several personality factors and their level of oxidative stress (serum MDA levels), as it is a well-known fact that medical science is both physiologically and psychologically demanding. Furthermore, it was considered that certain personality structure could be contributing to psychiatric disorders or acting as a coping technique. Sample size was calculated with the help of WHO sample size calculator, the population size was obtained from United Nation's population website taking total population aged between 15-25 years in Pakistan present 2022 which is 30% of 229M (68700000). The total minimum estimated sample size was 69, keeping confidence interval as 90% and margin of error as 10%.

The modified Satoh method was used to quantify the amounts of MDA, a consequence of lipid peroxidation. The activity of thiobarbituricacid (TBA) with MDA, one of the aldehyde products of lipid peroxidation, is the basis for this assay. 0.5 ml of serum was combined with a reagent containing 20% TCA and centrifuged for 10 minutes at 3,500 rpm. The precipitate was rinsed with 2 cc of 0.05 M H2SO4 and centrifuged for 10 minutes at 3,500 rpm. Three millilitres of TBA were added, and the tubes were placed in a boiling water bath for 30 minutes before being cooled under running tap water. For 5-10 minutes, four milliliters of n-butanol were vigorously shaken or vortexed. For 10 minutes, the sample was centrifuged at 3,000 rpm. The optical density of the supernatant fluid was measured in the spectrophotometer at 532 nm. The serum levels were determined using a standard graph. [11]

For psychological assessment, DASS (Depression, Anxiety, and Stress Scale) was used. [12]

Students of both genders between the ages of 18 and 25 who were nonalcoholic, nonsmokers, and non-hypertensive were included in the study. Students over the age of 25 and had diabetes, smoking habits, or drunkenness were excluded. Also, if they were not agreed upon for examination (biochemical/psychological), they were not taken for study. Throughout the tests, individuals were also asked if they had any complaints of biochemical/psychological problems.

Data was analyzed in three categories, first category assessed the demographic variables

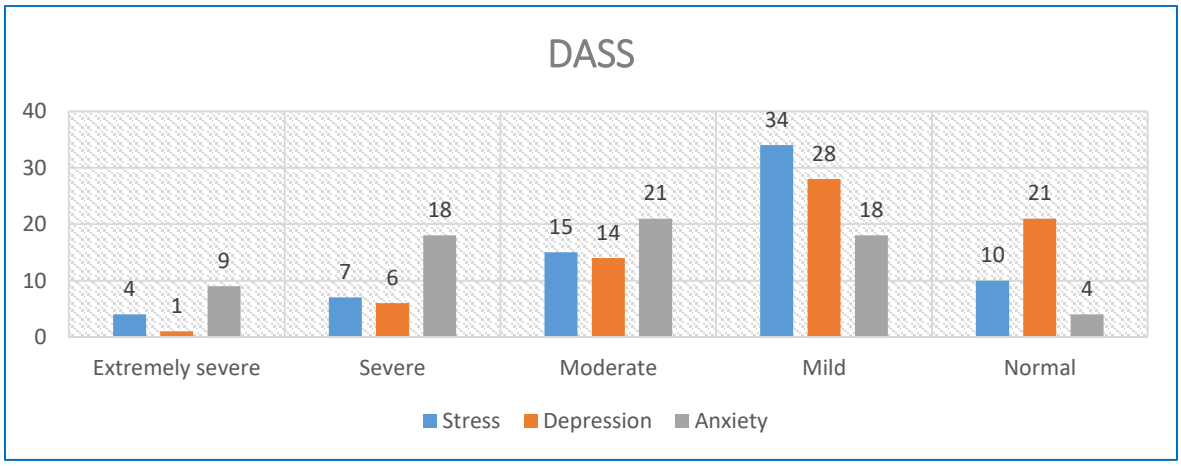
including age, gender, Basal metabolic index (BMI), socio-economic status and level of education. Second category assessed the level of serum MDA, while third category indicated the data from DASS score providing insight of depressive illness, stress and anxiety levels in respondents.

Statistical package of social sciences (SPSS) version 21 was used to analyze the data, independent variables such as age, BMI, Socio-economic status and gender distribution was analyzed with the help of frequency, percentage, mean and standard deviation test. MDA was analyzed with the help of mean and standard deviation while DASS was measured according to the pre described method and participants were assessed in frequency. Student t-test was used to assess comparison between groups, chi-square test was used to determine data significance with p-value less than 0.05.

Results:

Total 70 students participated in the study, with gender distribution of 48 (68.5%) females and 22 (31.4%) male participants. Mean age was 18.4 ± 4.8 years, further divided into three categories of 16-19 years, 20-22 years and 23-25 years. Dominate population was under the age of 16-19 years with 43 (61.4%), followed by 23-25 years with 16 (22.8%) and 20-22 years with 11(15.7%) participants respectively. Students were recruited from 1st year, second year and third year classes. Over all MDA mean value was determined as 3.05 ± 0.3 while depression, anxiety and stress scale results represented within extremely severe, severe, moderate and mild categories. DASS results indicated maximum numbers of anxiety symptoms as compared to stress and depression. 10 (14.2%), 21 (30%) and 04 (5.7%) participants were reported as normal in all stress, depression and anxiety respectively. (Fig 01)

Fig 01: Results from DASS score from total study population.



Upon comparing the groups as per educational difference and age groups, the MDA values were reportedly higher in first and second year, similarly study participants in age groups of 16-19 years and 23-25 years had higher levels of MDA as compared to other group. DASS scores were reported as higher in first year students while 16-19 years of age as compared to other groups indicating higher level of psychological issues in newly admitted students. Table 01

Variables		MDA (mean ± St.Dev)	Stress n (%)	Anxiety n (%)	Depression n (%)	P-Value
Educational year groups	First year	3.1 ± 0.26	35 (50%)	27 (38.5%)	17 (24.2%)	0.02
	Second year	3.2 ± 0.41	27 (38.5%)	21 (30%)	22 (31.4%)	0.18
	Third Year	2.9 ± 0.51	4 (5.7%)	18 (25.7%)	10 (14.2%)	0.04
Age groups	16-19 years	3.2 ± 0.18	27 (38.5%)	32 (45.7%)	24 (34.2%)	0.81
	20-22 years	3.1 ± 0.42	29 (41.4%)	22 (31.4%)	17 (24.2%)	0.01
	23-25 years	3.2 ± 0.81	8 (11.4%)	12 (17.1%)	8 (11.4%)	0.05

Discussion:

The primary source of stress in students is parental or family pressure to perform well and stand out from the crowd. Children may experience frustration, physical stress, anger, unpleasant complexes, and sadness if they are unable to satisfy that expectation, or if they are in the process of meeting it. Underachievers exhibit negative tendencies such as shyness, unfriendliness, and jealousy, and may withdraw into their own world to become loners. [13]

Overscheduling a student's life can also cause stress. A child's after-school and in-school activities should be carefully planned to allow them some breathing room. Parents may wish him to learn music, paint, or excel in a specific sport. [14]

Factors of physical, physiological, or psychological origin [15-19]

Physical symptoms of stress or tension include fatigue, muscle tension, palpitations (a beating heart or an increased heart rate), and headaches. Sweating (cold sweat) or hot flushes, shallow or erratic breathing, a feeling of being choked or smothered with pain in the chest, nausea or abdominal distress, feeling numb or experiencing tingling sensations in certain parts of the body, experiencing a dry mouth and the urge to swallow repeatedly, diarrhea, impotence or an excessive need for sex, asthma, dizziness, unsteadiness, lightheadedness, headaches, cons

Emotional aspects [20-24]

Feeling depressed and downhearted at times, feeling detached from oneself, fear of losing control or going insane, fear of dying, intense apprehension, fearfulness or terror (often associated with feelings of impending doom), increased irritability or anger, anxiety or feelings of panic, tearfulness, increased interpersonal conflicts, and so on are emotional factors indicating stress or tension.

Social Factors [25-27]

Challenges with family acceptance, not sharing with others, feelings of loneliness, difficulties with recreational activities, and so on.

Examination factors are conditions that induce stress in students that are directly or indirectly related to examinations, tests, academic or curricular activities, performances, or competitions.

Factors influencing behavior: [28-29]

Restlessness (feeling tense or on edge), trembling or shaking, short-temperedness, withdrawal from interpersonal interaction, excessive smoking, sleeping and/or drinking, sleep disturbances (finding it difficult to fall asleep or experiencing nightmares, sleeping excessively or disturbed sleep waking up tired), not feeling hungry or eating excessively, slow psychomotor coordination, rushing around, working longer hours are all behavioral factors of stress or tension.

Conclusion:

All groups have substantial levels of oxidative stress, but the senior group is significantly more stressed. There is a significant difference between the groups, with first-year students having higher levels of anxiety, stress, and depression. Within-group gender comparisons reveal no significant differences in any of the characteristics between boys and girls, not in the first, second or third years. Correlations between oxidative stress and DASS score disclose significant association between oxidative stress and Stress, anxiety, and depression. According to the foregoing findings, the MDA levels of second-year students are higher than those of first-year & third-year students, indicating a higher level of oxidative stress in second-year students. As a result, additional work on health enhancement can be done in this population.

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