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Research paper

Study of Stress and Depression Measurements Using HPLC as a Diagnostic Tool: Behavioural and Instrumental-Based Study

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ABSTRACT

Stress is a bizarre mental and emotional condition in reaction to bodily or physiological pressure. It is taken into consideration to be a primary element in inducing anxiety and depression which in turn can result in various health problems. It is regarded as activating the vital serotonergic gadget. Beneath physiological conditions, the level of serotonin (5-hydroxytryptamine: 5-HT) synthesis generally depends on the concentration of its precursor tryptophan (TRP). The physiological thing of stress alters the supply of TRP inside the brain and thereby affects the charge of 5-HT synthesis there. In this study, high-performance liquid chromatography (HPLC) was used as a diagnostic tool to monitor stress and despair responses in terms of TRP concentration. Because stress and depression are usually diagnosed through changes in a person's physical activity and behavior. Therefore, in addition to the instrument-based study, a behavioral study was also conducted. The prevalence of stress in the target population was studied on a biochemical and behavioral basis. The results of our study show that students who do not interact socially are more vulnerable to stress than students who do interact socially. Changes in TRP concentrations are suggested as an accurate and reliable biochemical basis for stress detection.

KEYWORDS: HPLC, Depression, Serotonin, Stress, Tryptophan.

INTRODUCTION

Stress or nervous tension performs a chief position in pathophysiological pathways associated with neurodegenerative sicknesses and mental problems [1]. In recent times nervous tension has grown to become out to be a part of our recurring lifestyles because the contemporary lifestyle is full of hassles, closing dates, frustrations, and needs. Strain is not always continually lifestyle-threatening. In small doses, it assists in carrying out under pressure and motivates [2,3]. Many behavioral changes can be observed in a stressed individual like disturbed eating and sleeping patterns, getting isolated from others, using addictive products to relax himself, etc. [4,5]. Serotonin (5-HT;5-hydroxytryptamine) is a monoamine neurotransmitter, biochemically derived from tryptophan, found in the gastrointestinal (GI) tract, platelets, and the central nervous system (CNS) of animals, including humans. Serotonin is involved in lots of capabilities like the urge for food, sleep, memory, mastering, muscle contraction, and cardiovascular features in addition to strain, despair, and nervousness. [6]. Increased level of 5-HT indicates the condition of stress whereas low 5-HT levels are the cause of mild to severe depression. Therefore, the 5-HT levels have been widely investigated as a key parameter in

the detection of stress which might lead to depression [7]. 5-HT levels have been widely investigated as a key parameter in the detection of stress which might lead to depression [8]. Tryptophan (TRP) is one of the 22 standard amino acids and a vital amino acid within the human regimen. It is far encoded within the well-known genetic code because of the codon UGG. The distinguishing structural feature of tryptophan is that it consists of an indole functional institution. It is the immediate pre-cursor to 5-HT [9]. Tryptophan is an amino acid that may be transformed into several important molecules together with serotonin and melatonin. Tryptophan and the molecules that it provides can affect many functions within the body which include sleep, temper, and behavior. An increased concentration of TRP is an indication of the condition of stress which might converge to depression [10]. Hence TRP serves as a fundamental marker of stress and depression states in an individual. Estimating the concentration of TRP thus facilitates monitoring stress & depression conditions prevailing in a person. To determine the concentration of TRP in blood samples, High-Performance Liquid Chromatography (HPLC) was used. In this study, HPLC acted as a diagnostic tool for monitoring stress & depression.

MATERIALS AND METHODS

Behavioral Study

In the first step, a literature review was conducted to gain maximum knowledge about what behavioral changes typically occur when a person suffers from stress. The target audience was selected after reviewing the literature. Healthy students from various institutions in Karachi were the target of this study and students with any chronic or hereditary disease were exempted from this study.

Based on the study, a questionnaire was designed which consisted of 25 questions to be filled in by the target students. Questions related to their daily routine may include their parenting, social interactions, social crises including academic or financial crises, proper food intake, and nicotine or tobacco addiction.

All contributors have been requested to post written consent for using the information contained in the questionnaire and for the donation of serum samples. After filling out the questionnaire, the information was analyzed the usage of "Pearson's Chi-Square test" which is used to investigate categorical statistics. Employing this statistical approach, a precise conclusion was reached in the end.

Equipment Based Study

In the first step, 50 blood samples were collected from the target students out of which 25 were "test" (living without proper parenting) and 25 "control" (living under proper parenting). Blood was allowed to clot at 4 ºC and serum samples were centrifuged earlier than being stored at -70 ºC until estimation of TRP.

In the second step, the mobile phase (buffer) was prepared. The mobile phase was composed of 1.56% Na_2SO_4, 10% methanol, and 0.005% EDTA in 0.1 M phosphate buffer of pH 2.9.

After preparation of the mobile phase, a standard solution including an L-TRP concentration of 2000 ng/ml was prepared, accompanied by an extraction medium to extract TRP from plasma along with 0.01 ml of plasma, 0.2 ml of 0.4 M perchlorate, 0.1 % sodium metabisulfite, and 0.01% EDTA had been delivered and combined thoroughly. The aggregate changed into then centrifuged at 12,000 rpm for 10–15 min at 4°C in an Eppendorf tube. The supernatants were then used for the

deduction of TRP by HPLC. Before analyzing the samples, the machine was calibrated using a separate widespread standard to become aware of its relative retention time.

Determination of Plasma TRP by HPLC-UV Method

High-performance liquid chromatography (HPLC) is likewise a form of liquid chromatography. For the 1970 Pitcon paper, the late Prof. The acronym HPLC, coined using Kasaba Horvath, first stated the reality that high pressure could be used to generate the flow required for liquid chromatography in a packed column. Since the characteristics of the sample composition may be different, many kinds of detectors have been developed. In our study, we used a UV detector. These detectors have a moderate sensitivity range down to approximately microgram quantities. In the first step, 50 blood samples were collected and centrifuged before storage. In the second step, the mobile phase (buffer) was prepared. The mobile phase was composed of 1.56% Na2SO4, 10% methanol, and 0.005% EDTA in 0.1 M phosphate buffer of pH 2.9. After preparation of the mobile phase, a standard solution containing an L-TRP concentration of 2000 ng/ml was prepared, followed by an extraction medium to extract TRP from plasma, which includes 0.01 ml of plasma, 0.2 ml of 0.4 M perchlorate, 0.1 % sodium metabisulfite, and 0.01% EDTA had been introduced and mixed very well. The combination was then centrifuged at 12,000 rpm for 10–15 min at 4°C in an Eppendorf tube. The supernatants were then used for the determinations of TRP by HPLC. Before analyzing the samples, the device changed into calibrated the usage of a separate standard to perceive its relative retention time. After acquiring the usual peak, the sample changed into loaded and its top turned into obtained.

Statistical Analysis

Recorded data were analyzed using one-way ANOVA. Post-hoc comparisons were made with the aid of the Newman-Keuls test; $p<0.05$ was taken into consideration.

RESULTS

The effect of regular exercise, proper food intake, social interactions, and appropriate parenting on stress dependence are shown in Figure 1-4 respectively. A good-sized reduction in strain dependence changed into shown within the test groups as compared to the controls in step with the Chi-square test. Moreover, exposure to social crisis (Figure 5) confirmed a full-size growth in stress dependence.

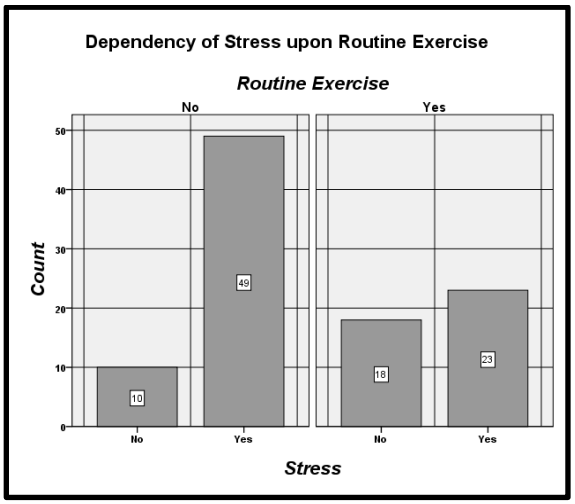

Figure 1: Dependency of Stress on Routine Exercise

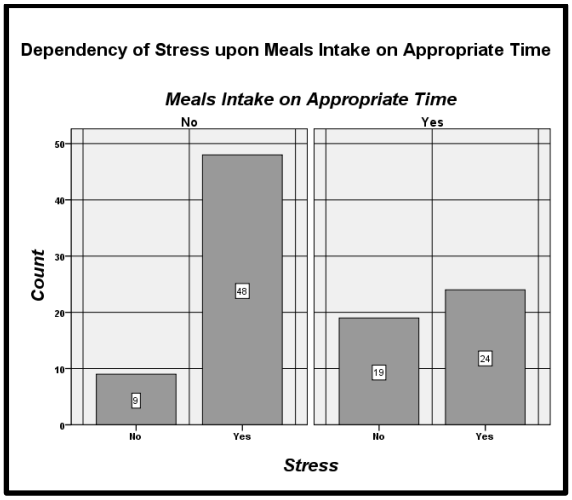

Figure 2: Dependency of Stress on Meals Intake on Appropriate Time

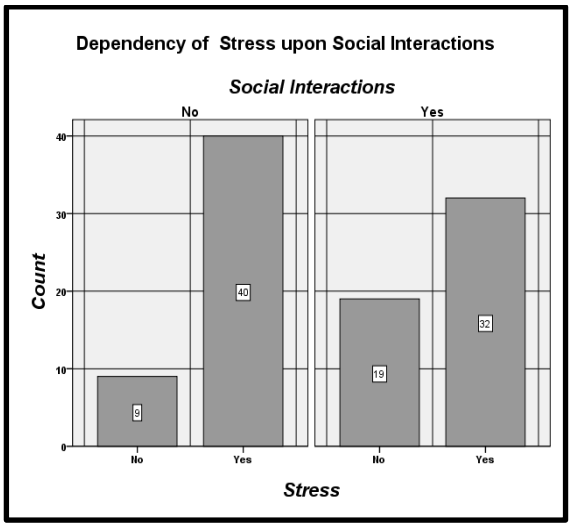

Figure 3: Dependency of Stress on Social Interactions

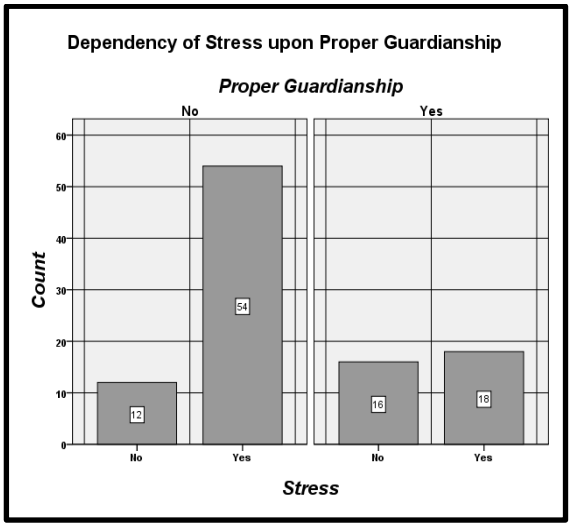

Figure 4: Dependency of Stress on Proper Guardianship

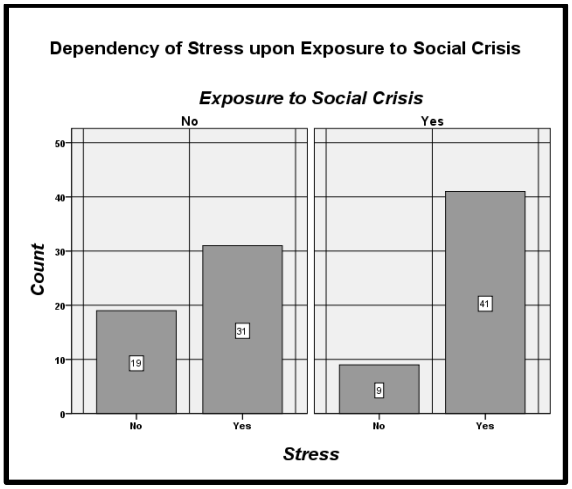

Figure 5: Dependency of Stress on Exposure to Social Crisis

The effects of strain on plasma TRP levels are shown in Figure 6. The results of our instrument-based study showed that stressed students had higher plasma TRP levels than non-stressed students with lower plasma TRP levels.

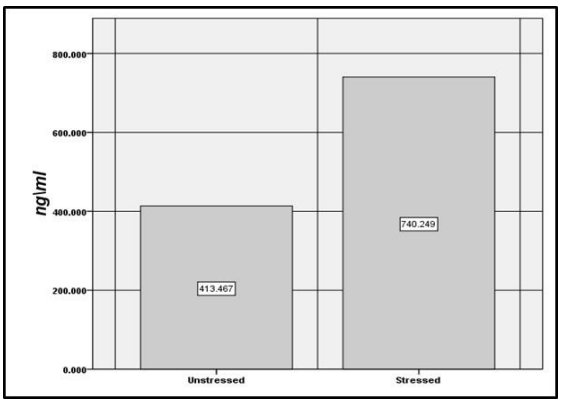

Figure 6: Mean TRP Values of Stressed and Unstressed Students

DISCUSSION

Stress is an inevitable part of life. Each day of exercise has been confirmed to have useful consequences on someone's intellectual and bodily states. According to the Anxiety and Depression Association of America (ADAA), the workout is also taken into consideration as critical for preserving mental fitness, and it can reduce

strain. Existing tools and vogue diets have given rise to a large look of régime-related sicknesses, which may be easily dealt with and averted with regular bodily activity [11,12]. According to the literature, regular participation in aerobic workouts has been proven to lessen standard stages of pressure, increase and stabilize temper, improve sleep, and enhance self-esteem [13,14]. Chronic exercising reduces oxidative stress produced by acute exercise [15–18]. The normal bodily workout provides systemic useful results, which include an improved physical feature, reduced disorder prevalence, and better high quality of existence [19,20]. It is pretty clear from the results of our survey that scholars who have an addiction to everyday workouts or take part in extracurricular activities like sports are less probably to be burdened than people who do not workout day by day. Biologically, exercising offers the body a danger to practice managing stress.

Many research studies have found that individual variations such as emotional ingesting and dietary restraint affect the relationship between pressure and ingesting. However, little research examines whether people are differentially privy to how much they devour throughout stressful situations. Results confirmed that emotional ingesting and strain have been related to the overestimation of overall food intake, whilst nutritional restraint in part mediated the relationship between pressure fame and actual meal consumption [21–23].

Eating at the right time is mandatory to maintain a healthy life. In stressful situations, the tendency to skip meals is especially noticeable among students. For example, have you ever found yourself rushing out of the house without a healthy breakfast (not counting late pick-ups)? Self-reported consequences of stress on consuming conduct behavior and ingesting were assessed. A typical increase in snacking at some stage in stress was pondered by using reports of improved consumption of "snack-kind" foods amongst all respondents, no matter dieting repute. In evaluation, a decrease in the consumption of "diet-type" meals was said at some point during traumatic durations [24]. In one observation, the consequences of exposure to persistent stressors of diverse intensities and everyday periods on food intake were tested in adult male rats [25]. The study concluded that prolonged exposure to positive intensities prompted anorexia and reduced overall frame weight in rats. Managing (low strain) had little effect on meal consumption and weight, at the same time restraint (slight strain) slightly reduced food consumption and weight, and restraint (excessive pressure) brought about extra severe reductions in meal intake and weight. Laboratory research displays mixed results because of the constraints of inducing pressure on human beings in the laboratory and the ability to measure nutritional consumption. The effects of our study a look at display that students who devour at the proper time (although a healthful meal must be eaten 3 instances a day which includes breakfast, lunch, and dinner) are much less likely to be confused than students who do not consume on proper the time.

Social interplay and mental well-being had been counseled to be critical in retaining exceptional lifestyles. A previous study suggested that topics felt more social aid after interacting with a friend than with a stranger. They had been given the impression to be much less depressed and more self-assured [26]. Although the effect of social interaction on individuals' mental fitness is not clean quantitatively, in a have a look that focused on the connection between observable social interplay and

mental health, researchers cautioned the possibility that people who reveal in extra social interplay tend to have much less stress [27].

Preceding studies defined social interplay as face-to-face contact time [2]. Their consequences advocate the possibility that people's social context may be assessed from their bodily physical interest styles. It was discovered that the distribution of resting periods of human physical interest follows a power law. [28]. Additionally, they located that the scaling exponent of the rest period distribution of depressed sufferers changed into a statistical decrease than that of wholesome topics. In a stressful situation, students avoid social interactions (i.e. interactions with family, friends, relatives, co-workers, etc.) unlike in a stressful situation. The results of our study also show that students who do not interact socially are more vulnerable to stress than students who do interact socially. Moreover, the results of our study show that students who face social crises (academic plus financial crises) are more likely to be stressed than students who have social crisis coping skills. The economic crisis is predicted to produce secondary mental fitness results that may boom suicide and alcohol deaths (WHO). Rising income inequality is linked to growing rates of suicide [29].

Giant studies demonstrate that own family environment is associated with strain. Formative years stressors affect youth and adolescent improvement, in the long run leaving the body vulnerable to ailment as a group [30]. Our study results show that students who are under proper guardianship (living with parents, friends, or relatives) have lesser chances of being stressed than those who do not have proper guardianship. The family structure presents a picture of children's living preparations, however, exhibits little approximately family experiences throughout early life. As youngsters are increasingly dispersed across a variety of circles of relative structures, several of which are greater stable than others, it’s miles critical that researchers explicitly don’t forget the dynamics of own dwelling family arrangements across early life. Family balance is as important for an infant's proper being as a circle of relative shape and has on-the-spot and long-time period advantages for kids [30-34]. As an example, a circle of relative stability at some stage in excessive school has been connected to younger growing consequences, which include excessive school commencement, university enrollment, smoking, and consuming, and sexual initiation [35]. Similarly, Wu and Thomson (2001) [36] showed that the circle of relative transitions, in preferences to extended exposure to a single mother or the prolonged absence of a biological father, had been associated with early sexual initiation. Albrecht and Teachman's [37] studies showed this location; the variety of transitions was related to the threat of first premarital intercourse. For other domains names of well-being, (e.g., externalizing behaviors, cognitive consequences), the circle of relative structure turned into children's outcomes internet of own family instability [38,39], which illustrates the utility of thinking about both own family shape and balance.

Exercise may improve intellectual health by assisting the mind to cope more with strain, following investigating of the effect of exercising on neurochemicals worried inside the body's strain reaction. Whilst strain influences the mind, with its many nerve connections, the rest of the body feels the effect as well. So, it stands to motive that if your body feels higher, so does your mind. Many researchers have reported that

acute depletion of TRP occurs in people habitual of having TRP-free drinks in their routine which causes a reduction in mind serotonin stages as well as depressed temper, mainly in ladies suffering from a private or familial history of depression [40-45]. The results of our equipment-based study indicate that the plasma TRP levels in stressed students are high in comparison to the unstressed students which have low plasma TRP levels. Hence change in the concentration of TRP serves as an accurate & reliable biochemical basis for the detection of stress**.**

CONCLUSION

This survey and instrument-based research will provide a better understanding of the behavioral and physiological changes associated with stress and also provide some healthy key points that will help the younger generation avoid and cope with stress-related defects in their lives, as the younger generation, especially the students. They are the building blocks of any society. In addition to academic activities, a healthy, stimulating, and enriching environment will help them contribute to making society a successful place to live in, not only academically but also in their professional fields.

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