**ZINC AND TRYPHTOPHAN LEVELS IN ANOREXIA NERVOSA; A CO-RELATIONAL STUDY**

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**ABSTRACT**

Anorexia nervosa (AN) is a condition in which an individual possess low body weight, obsession with having a thin figure, fear of gaining weight and inappropriate eating habit. It is often coupled with a distorted self-image. Irrational fear of gaining weight and immoderate eating habits may cause it. People suffering from anorexia nervosa have low leptin level and high tryptophan levels. Tryptophan and zinc levels have their eminent effect on anorexia nervosa. Tryptophan synthesizes serotonin, in brain neurons and stored in vesicles. Serotonin (5 hydroxy-tryptophan--5-HT) is a neurotransmitter in the brain that has an enormous influence over many brain functions, involving appetite control. Zinc is involved in numerous aspects of cellular metabolism. Zinc daily intake is required to maintain a steady state as no specialized zinc storage system has found in body. Tryptophan and zinc serum levels were determined in 40 subjects out of which 20 were suffering from anorexia nervosa and 20 were normal, blood samples were centrifuged to extract plasma serum. Plasma tryptophan levels were examined in subjects by using HPLC (high performance liquid chromatography), whereas zinc level were examined in plasma serum using FAAS (flame atomic absorption spectroscopy). BMI of the subjects was kept within the range of 19.00kg/m².

It was observed that the tryptophan levels were high and zinc levels were low in subjects suffering from anorexia nervosa at all times.

**KEYWORDS**

Anorexia nervosa (AN), Tryptophan, Serotonin (5HT), Zinc, Body mass index (BMI), FAAS, HPLC.

1. **INTRODUCTION**

Anorexia nervosa is an eating disorder characterized by immoderate food restriction and irrational fear of gaining weight, as well as a distorted body self-perception [1]. It is a complex sort of eating disorder in which refusal to maintain a healthy body weight is found. Also, distorted body image and an intense fear of gaining weight are considered its symptoms. Adolescents suffers anorexia nervosa the most and the ratio of female adolescent anorexics is higher than the male ones [2, 3]. Causes of anorexia could be psychological, social, it could be caused by media effects, relationship to autism, malnutrition etc [4]. Individuals suffering from anorexia nervosa may exhibit several signs and symptoms, severity and type of which may vary case to case and may be present but not readily apparent. Severe complications are caused due to self-imposed starvation associated malnutrition and anorexia nervosa in every major organ system in the body [4-6]. A typical anorexic patient shows symptoms like low BMI, depression, mood swings, amenorrhea, extreme dieting, fatigue etc [7-10]. There are two types of anorexia, restricting anorexia and
purging anorexia. Weight loss in restricting anorexia is due to restricting calories (following fasting, drastic diets and exercising to excess). Weight loss, in purging type of anorexia is achieved by using laxatives and diuretics or vomiting [11]. Our study examines the plasma serum levels of tryptophan and zinc in anorexic patients.

The ratio of plasma tryptophan predicts uptake of tryptophan by brain and serotonin production. If this ratio is low in subjects, serotonin function may also be low [12]. Due to lack of tryptophan, which is responsible for production of serotonin, it has been observed that underweight patients with anorexia nervosa have reduced serotonin activity [13, 14]. Low availability of tryptophan to the brain indicates that ratio of tryptophan to the large neutral amino acids is low [15]. Serotonergic neurons regulate the food intake [16]. L-tryptophan, amino acid precursor, controls synthesis of serotonin in the brain [17]. Hence, treatments that upgrade production of serotonin reduced intake of food, whereas those that abates serotonin synthesis stimulate intake of food [18]. Regulation of tryptophan affects appetite and serum tryptophan ratios are above normal in our selected subjects therefore their food consumption is less than that of a normal person [19]. The current study concludes that tryptophan levels are high in anorexics despite of the weight loss [20]. Eating disorders has been found associated with zinc deficiency, studies suggest that anorexic individuals loose the sense of smell and taste which is associated to zinc deficiency. Increased risk of coronary heart disease, eczema, gum inflammation, diabetes, tinnitus, poor wound healing and acute diarrhea in Third World children [21]. Zinc deficiency is seen in adolescents. We can suggest that zinc status can be compromised by zinc intake [22, 23]. Anorexic patients have many attributes of zinc deficiency, skin abnormalities poor growth or weight loss, depression and amenorrhea [24]. Zinc supplementation is required to overcome anorexia nervosa [25]. The aim of the conducted study is to co-relate the levels of zinc and tryptophan in plasma with anorexia nervosa, and to evaluate whether they are un substantial or normal and what effects they have on anorexic subjects.

2. MATERIALS AND METHODS

2.1. Anthropometric Measurements

Body mass index (BMI), calculated as the body weight divided by the square of the height (kg/m²), was used as a marker of obesity. Weight and height were measured. Patients were designated as anorexic when BMI was below 20 kg/m² and were designated normal when BMI was above then 21 kg/m².

2.1.1. Subject & Study Design

To evaluate our study a questionnaire was given to the selected subjects to be filled out. On the basis of their answers they were separated into anorexics and non-anorexics also. Blood samples were taken from 40 random volunteers whose serum was extracted using centrifuge machine. Samples were taken without any particular criteria under normal homeostatic conditions with no fasting. Then it was used to find out the tryptophan levels and zinc levels in sample.

2.2. Biochemical Analysis

Two techniques are used for our research purpose. HPLC (high performance liquid chromatography) for finding out tryptophan levels and FAAS (flame atomic absorption flame spectrophotometry) for zinc level determination.
2.2.1. HPLC (High Performance Liquid Chromatography):

Plasma samples were extracted and the levels of Serum tryptophan were determined by HPLC-EC [26]. A Shim-Pack ODS of 5 µm, 4.0 mm internal diameter and 150m.m. length of separation column was used. Mobile phase containing octyl sodium sulfate (0.03%) and EDTA (0.0035%) in phosphate buffer 0.1M of pH 2.9 at an operating pressure 2,000–3,000 psi on Shimadzu HPLC pump separated the sample. Shimadzu L-ECD-6A detector at an operating potential of 0.6 V undergoes electrochemical detection. Plasma samples 0.2 um/ml and extraction medium 0.01um/ml was taken to run a test on HPLC to find out plasma tryptophan levels in subjects suffering from anorexia nervosa.

2.2.2. FAAS (Flame Atomic Absorption Spectrophotometry)

Metals and metalloids are commonly detected through Flame atomic technique. It is based on the fact that metals at ground state absorb light at specific wavelengths. In a solution by means of a flame metallic ions are converted to atomic state. Light of the appropriate wavelength of 213nm is supplied and the amount of light absorbed. Flame atomic absorption spectroscopy is used for the determination of zinc concentration in human blood [27].FAAS works on the principle of absorption of light. The hollow cathode lamp is responsible for the production of a spectrum of a specific wavelength. Sample is converted into population of free atoms by the atomizer. The light then passes through the free atoms population. The detectors then detect the transmitted light. To find out zinc levels 0.1 mg/ml of sample and remaining 0.9 mg/ml of standard solution were taken and results showed the zinc level in subjects suffering from anorexia.

3. STATISTICAL ANALYSIS

Results expressed as mean +/- standard deviation (S.D). Statistical analysis was determined by paired sample T test. Statistical analysis included standard methods for comparison between variables. The p-values <0.05 was considered as a significant. IBM-SPSS 20 is used for all statistical analysis.

4. RESULTS

Figure 1 shows the effect of anorexia nervosa on zinc levels. Paired sample test showed that the difference between zinc levels of anorexic and non-anorexic people are significant (P<0.05).

![Zinc Level](image)

Figure1. Zinc level in anorexic and non-anorexic subjects
Figure 2 shows the effect of anorexia nervosa on tryptophan levels. Paired sample test showed that the difference between tryptophan levels of anorexic and non-anorexic people are significant (P<0.05).

5. DISCUSSION

Differences between tryptophan and zinc levels were primarily observed in anorexic subjects on molecular basis. Through our result we have concluded that anorexic individuals have increased plasma tryptophan levels and decreased levels of zinc. According to American Psychiatric Association (1994), anorexia nervosa most commonly occurs in adolescent girls. Anorexia nervosa is found as the leading cause of death in young females 15 to 24 years of age, it has the highest mortality of any psychiatric diagnosis. Death occurs due to suicide, infections or succumbing to the effects of chronic starvation [28]. Unusual patterns of feeding behavior and weight regulation is observed anorexic individuals. It is characterized by an uncomplying obsession with fatness and fear of eating and weight gain, even having bright chances of increasing cachexia [29]. Mostly individuals suffering from anorexia possess certain psychological factors that describe their personality, and they have tendency towards anxiety and depression [30]. The etiology of AN is complex, evidence states that genetic predisposition, premature births, birth trauma and bio-chemical individuality may also play an important role in the eventual development of anorexia nervosa [31]. The eventual development of Tryptophan is an essential amino acid which is received and sustained in body from diet. The body uses tryptophan to help make niacin and serotonin our edict consistent to previous researches appraises that any diversity in tryptophan levels effects the production of serotonin [32]. Recent studies have raised the question as to whether a disturbance of the serotonin (5-HT) neurotransmitter system could contribute to behavioral alterations in AN. It is well known that brain 5-HT systems contribute to the modulation of appetite; our pronouncement determines the effect of high levels of tryptophan on responsiveness of satiety [33]. Serotonergic neurons can either increase or diminish the diet. An increase of intra synaptic 5-HT tends to reduce food consumption [34, 35]. Insulin resistance, which may be present in anorexia, impairs the body’s ability to produce serotonin from L-tryptophan [36]. Acute dieting and weight loss can cause low levels of plasma L-tryptophan [37]. Thus, increased brain 5-HT activity could play a role in enhanced satiety in AN. In addition, people with AN tend to be anxious, obsessive, perfectionist, and harm avoidant [38].
We studied the association of zinc deficiency and anorexia nervosa because the relationship is controversial [39]. Zinc is involved in numerous aspects of cellular metabolism [40, 41] and it plays a role in immune function, protein synthesis [41, 42-43]. Zinc deficiency has been found to be a common feature in anorexics, symptoms in common which include poor appetite, weight loss, nausea and amenorrhea. It is consistent with previous studies that zinc deficiency may actually play a role in the causation of anorexia nervosa [44]. Low zinc levels, appear to be secondary to self-starvation, and are rapidly reversible without zinc supplementation [45]. In our findings results reveals tryptophan levels were high in anorexics and zinc levels were low. The clinical manifestations of zinc deficiency and anorexia nervosa are remarkably similar, and a number of studies have demonstrated that there may be a positive correlation between zinc therapy and the rate of recovery of anorexia nervosa patients. However, because of the different interpretations, the use of zinc supplementation varies. Zinc supplementations in the treatment of anorexia nervosa have a positive effect on recovering. Zinc therapy enhances the rate of recovery in anorexia nervosa patients by increasing weight gain and improving their levels of anxiety and depression. Low levels of zinc shows that zinc supplementation should be included in treatment protocol to treat the eating disorder [46]. Dietary concentrations of tyrosine and tryptophan could affect the synthesis and concentrations of the neurotransmitters nor-epinephrine and serotonin which in turn, influence central concentrations of these neurotransmitters which affects the relative appetite/satiety state of an individual [47]. These findings spurred nutrition researchers to connect zinc deficiency, dietary amino acid intake and anorexia [48].

Because of the host of complications anorexia causes, you may need frequent monitoring of vital signs, hydration level and electrolytes, as well as related physical conditions [49]. There are no medications specifically designed to treat anorexia because they haven't been found to work very well. However, antidepressants or other psychiatric medications can help to treat other mental disorders you may also have, such as depression or anxiety [50].

CONCLUSION

According to the present study and results it is clear that anorexic people have high tryptophan and low zinc levels in their body then a normal person. These high levels of tryptophan and low levels of zinc triggers anorexia nervosa as tryptophan is a precursor of serotonin which is a mood regulating neurotransmitter its enhanced levels can cause decreased eating which results in anorexia nervosa, also it’s a mood regulating neurotransmitter and its unsubstantial levels can cause depression. Low levels of zinc in volunteers suggests that they are zinc deficient which resembles those of anorexia nervosa to some extent. This has led our research to theorize that low zinc levels and high tryptophan levels can be related to the onset of the eating disorder anorexia nervosa.

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