RESEARCH ARTICLE

MICRONUTRIENTS AND OXIDATIVE STRESS IN ADOLESCENT GIRLS.

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Manuscript Info	*Abstract*
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Background: Nutritional anemia is a disease caused by malnutrition in its widest sense. The most frequent cause of nutritional anemia is iron deficiency. Adolescent is an intense anabolic period where requirement for all nutrients increase. Poor nutritional status causes impairment of the antioxidant defense system and reduced cellular immunity were previously reported in patients with IDA.
Aims and Objectives: to determine the level of micronutrients, antioxidants and marker of lipid peroxidation and their correlation among anemic and non-anemic adolescent girls.
Material and Method: In the present study enrolled total 90 randomly selected school going adolescent girls, which were divided into two groups viz. non anemic (n=20) and anemic (n=70), group two further divided into two groups on the basis of degree of anemia viz. mild (n=30) and moderate (n=40) anemic. We measured complete blood count, levels of micronutrients, plasma lipid peroxidation product, enzymatic and non-enzymatic antioxidant. **Result:** The level of complete blood count, serum iron and zinc along with enzymatic antioxidant SOD were significantly reduced in anemic group. However, the levels of MDA, a marker of lipid peroxidation were found to be significantly increased in anemic group as compared to non-anemic adolescent girls. Non enzymatic antioxidant vitamin C also decreased significantly in anemic group.
Conclusion: nutritional iron deficiency anemia tends to increases the pro-oxidant components, which may results in various complications including peroxidation of vital body molecules resulting in increased adverse effect on educational performance, productivity and wellbeing.

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Introduction:-
Anemia is one of the most common nutritional deficiency diseases observed worldwide [1] and about 50% of Indian population is anemic. Numerous studies on adolescent girls have reported that the incidence of anemia ranges from 22.00 to 96.50% in India [2]. Nutritional anemia is, in its broadest sense, a disorder triggered by malnutrition. It has been defined by the World Health Organization (WHO) as "a scenario where the blood hemoglobin content is lower

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than usual owing to the deficiency of one or more essential nutrients, regardless of the cause of such impairment." The most prevalent cause of nutritional anemia is iron deficiency and less frequently folate and B12 deficiency [3]. Iron-deficiency anemia is a severe public-health concern in most developing countries [4].

Iron is vital in all living cells; about 75% of complete body iron is connected with hemoglobin responsible for oxygen transport [5]. Deficiencies of essential nutrients remain a worldwide issue, particularly in developing nations among females and children [6]. The contributing factors that are accountable for iron deficiency anemia are lack of money, poverty, low bioavailability of iron from cereal-based diet, poor nutritional habits, poor hygiene and sanitation that increase the risk of diseases such as hook worm infestation. These infections further increase the risk of anemia [7]. In India, the number of adolescents, especially girls, lives in suboptimal circumstances characterized by poor dietary status and elevated morbidity and mortality levels [8]. This scenario becomes even more complex when adolescents are often subjected to diseases and parasites that may compromise their dietary status. One way to break the cycle of intergenerational malnutrition is to improve nutrition for adolescent females for preconception. If the vicious cycle of malnutrition will continue lead to ever more severe consequences. Micronutrients collectively referred as; vitamins and minerals that are vital for ordinary human development and functioning and are required in minute quantities [9]. Vitamins also have a significant impact on adolescent girls ' health and development. Vitamin C helps to absorb iron by maintaining ferric ion in the ferrous state, thereby facilitating absorption due to vitamin C reducing properties. Folic acid and vitamin B12 are also that play a significant role in keeping normal level of hemoglobin [10]. In patients with iron deficiency anemia, the literature provides contradictory and limited information on oxidative stress and antioxidant defense. Thus the present study is aimed to determine the, complete blood cell count, serum iron, and zinc status in randomly selected school going adolescent girls and correlate them with oxidative stress.

Material and Methods:-
The present study was carried out in the department of Biochemistry, ethical clearance obtained from Institutional Ethics Committee, MGM Institute of Health Sciences, Navi Mumbai. Study includes total 90 randomly selected adolescent girls aged between 10-19 years was selected from rural area of district Pune. Of which 20 healthy non-anemic and 70 was anemic adolescent girls [Mild anemia n=30 and Moderate anemia n=40] from low to middle socio economic status. The Anemic subject was according to WHO criteria, which defines mild anemia as Hb 10-11.9 gm/dl, moderate as Hb 7.0-10.9 gm/dl and severe anemia less than 7.0 gm/dl for adolescent girls. Exclusion criteria Metabolic disorder like Diabetes mellitus, Heart disease, Endocrine disorders, having infection such as Tuberculosis, HIV and Malignancy and any other systemic disorder. The anemia was diagnosed by estimating Complete Blood Cell Count on fully automated sysmax cell counter, serum Iron, TIBC was estimated by standard kit method. The antioxidant Vitamin C and serum Superoxide Dismutase (SOD) and marker of lipid peroxidation is serum Malondialdehyde level (MDA) was estimated by standard spectrophotometric methods. The statistical analysis was done by on SPSS version 20 with 95% confident interval. A pre-tested and pre-designed proforma was used to collect the information on socio-demographic characteristics like age, education status, family size, monthly income; medical history like age at menarche, history of worm infection, excessive menstrual bleeding in the past 3 months and dietary history. Blood sample was collected under aseptic condition after taking consent of participant or her parents by filling a consent form. Total 10.0 ml blood was collected, of which 2.0 ml of blood in EDTA vaccutener for determination of Hematological parameters, 3.0 ml in Heparinized vaccutener for estimation of plasma vitamin C level and 5.0 ml in plain vaccutener for analysis of biochemical parameters. Statistical analysis was performed on SPSS version 20 and Chi-square test and independent t test were applied.

Results:-
Table 1: Showing Distribution of Adolescent Girls.

Adolescent Age Group [in years]	Non Anemic Adolescent Girls [Group-1]	Anemic Adolescent Girls [n=70] [Group-2]	
		Mild Anemic [Group-2a]	Moderate Anemic [Group-2b]
10 to19	n=20 [22.2%]	n=30 [33.3%]	n=40 [44.4%]

The above table shows randomly selected total 90 Adolescent Girls, which were divided into two groups on the basis of degree of anemia. Group-1 includes 20 non anemic and group-2 includes 70 anemic adolescent girls, group-2 further divided into two groups. The group-2a includes 30 mild anemic and group-2b consists 40 moderate anemic adolescent girls.

Table 2:-Showing Hematological Parameters in Non-Anemic and Anemic Adolescent Girls.

Hematological Parameters	Non anemic Adolescent Girls	Anemic Adolescent Girls	
		Mild Anemia	Moderate Anemic
Hb [gm/dl]	12.8±0.5	10.6±0.4**	8.7±0.87**
RBC [million/cumm]	4.2±0.2	4.0±0.4 NS	3.6±0.8*
PCV [%]	37.1±1.6	32.4±1.3**	27.3±3.1**
MCV [fl]	85.9±4.2	80.5±6.7*	76.1±10.5**
MCH [pg]	29.7±1.8	26.5±2.7**	24.9±4.5**
MCHC [gm/dl]	34.7±1.5	32.8±1.2*	32.5±2.0*
RDW [%]	13.5±1.3	15.3±1.5 *	16.5±3.7**
Serum Iron [µg/dl]	95.4 ± 14.8	80.4 ± 11.4**	75.3±7.0**
Serum TIBC [µg/dl]	300.7 ± 33.1	353.4 ± 39.9**	400.33±18.5**

Results are presented as mean ± S.D. NS *p>0.05*, S* p<0.05, HS **p<0.001
The table 3 indicates levels of hematological parameters. The mean values of Hb, PCV, RBC indices and serum iron were significantly decreased ware as serum TIBC and RDW was significantly increased in mild and moderate anemic girls as compared to non-anemic adolescent girls.

Table 3:-Showing Biochemical Parameters in Non-Anemic and Anemic Adolescent Girls.

Biochemical Parameters [Units]	Non Anemic Adolescent Girls [n=20]	Mild Anaemic Adolescent Girls [n=30]	Moderate Anemic Adolescent Girls [n=40]
Serum MDA [nmol/ml]	2.3± 0.38	2.9 ± 0.58*	3.6±1.6**
Plasma Vitamin C [mg/dl]	1.1± 0.4	0.89 ± 0.30*	0.75±0.08**
Serum SOD [Units/ml]	4.9 ± 0.3	4.37 ± 0.4**	3.8±0.47**
Serum Zinc [µg/dl]	88.9 ± 8.9	79.1 ± 8.2**	68.7±10.4**

Results are presented as mean ± S.D.   S**P<0.05*, HS***P <0.001*

The table 3 shows levels of serum MDA, SOD, Zinc and plasma Vitamin C in non-anemic, mild and moderately anemic adolescent girls. The mean value of MDA was significantly increased in mild and moderately anemic as compared to non-anemic adolescent girls. Whereas levels of serum SOD, Zinc and plasma Vitamin C was significantly decreased in mild and moderate anemic as compared to non-anemic adolescent girls.

Table 4:-Showing Correlation of Hb with lipid peroxidation product in Moderate Anemic Adolescent Girls:

Parameters	Hb [gm/dl]	Serum MDA [nmol/ml]
Serum Iron [µg/dl]	r = 0.81	r = - 48
Serum TIBC [µg/dl]	r = - 0.79	r = 0.33
Plasma Vitamin C [mg/dl]	r = 0.70	r = - 0.34
Serum SOD [Units/ml]	r = 0.71	r = - 0.40
Serum Zinc [µg/dl]	r = 0.66	r = - 0.36

Discussion:-
In the present study we determined levels of complete blood cell count, serum Iron, TIBC, MDA, Zinc, Vitamin C and SOD activity in school going adolescent girls. We have studied prevalence of anemia and correlation of IDA with oxidative stress in study groups. Adolescent school girls were chosen as the subjects for this study because these girls are likely to enter in child bearing phage. Their growth during adolescence will affect their ability to carry a successful pregnancy and thus determine the well-being of the next generation. Severe nutritional deficiencies of one or more hematopoietic factors typically result in anemia from iron and less commonly from folate or B12. Iron

deficiency anemia is one of the world's most prevalent nutritional disorders, particularly in India and other developing countries [11].

We also found that prevalence of anemia was 77.7% in adolescent girls, of this 33.3% were mild anemic and 44.4% were moderate anemic; however we could not find severe anemic girls. Among the 77.7% anemic adolescent girls, 40.4% were suffering from hypochromic microcytic anemia. We observed positive correlation (r = 0.81) between hemoglobin and iron and negative correlation (r = -0.48) between serum MDA and serum iron level in moderately anemic adolescent girls. Our study in accordance with S. Kaur et al. identified epidemiological correlation of nutritional anemia in Rural Wardha among adolescent women and their results were 59.8 percent incidence of anemia, as well as significant correlation between anemia and vegetarian diet, excessive menstrual bleeding, iron consumption and worm infestation [12].

The information collected on the basis of questionnaire from adolescent girls, that reveals most of the adolescent girls having mild to moderate degree of anemia belonged to low to middle socio-economic class with lack of proper nutrition and poor quality of life style. We observed most them having, tea, chapattis, pickles and very few vegetables which were relatively economic. In 40.4% of anemic adolescent girls, the IDA is characterized by hypochromic, microcytic anemia. It may be due to lower iron availability for Hb synthesis, which is the required for RBC maturation. Defective Hb synthesis affects RBC maturation resulting in hypochromic, microcytic anemia.

In our study we observed the decreased level of antioxidants like Zinc, SOD and Vitamin C with significant increase in lipid peroxidation [MDA] level in anemic as compared to non-anemic adolescent girls. Increased lipid peroxidation and decrease in antioxidant defense enzymes causes oxidative stress [OS] with IDA. We observed negative correlation of serum MDA with Vitamin C, SOD and Zinc (r = - 0.34, r= - 0.40, r = -0.36 resp.) and positive correlation between serum MDA and TIBC (r = 0.33) in moderate anemic adolescent girls.
Zinc also plays a significant role in the synthesis of hemoglobin by activating delta-aminolaevulinic acid (δ ALA) dehydrogenase; it is an enzyme essential for the formation of porphobilinogen from two ALA molecules. Zinc and iron are vital trace elements needed to activate certain antioxidant enzymes, such as superoxide dismutase [CuZn-SOD], a main free radical scavenging enzyme [13].

Probably by enhancing zinc solubility and bioavailability, vitamin C improves zinc absorption. Vitamin C is also a powerful antioxidant and reduction agent that directly reacts with superoxides, hydrogen peroxyl radicals, and verious lipid hydroperoxides. It can also restore the oxidized vitamin E's antioxidant characteristics [13].

In many enzymes such as SOD, catalase, etc., iron acts as a cofactor. These are free radical scavenging antioxidant enzymes. Because of iron deficiency, the activity of these enzymes may be impaired by increasing the rate of free radical generation causing increased oxidative stress. Decreased Vitamin C, SOD and Zinc levels and consequently increase in MDA levels in IDA may be linked to increased oxidative stress, because it is well known that ROS, especially hydrogen peroxide (H_2O_2), inhibit SOD activity [14]. Anemia has a wide range of clinical consequences; one of the consequences of severe ID is a reduction in the life span of RBCs. This can be ascribed to a rise in the rigidity of the membrane and a decline in the causes of deformability to boost oxidative stress [15]. Therefore we **concluded** that nutritional iron deficiency anemia during adolescent age is associated with deficiency of essential micronutrients and free radical generation, which can cause peroxidation of vital body molecules that, leads to increased risk of adverse effect on their reproductive life if it is not treated well in time. The present study highlights the need to develop pragmatic intervention programs incorporating various strategies at school level to improve dietary intake and awareness towards importance of health. The combination of nutritional education, health awareness, and micronutrient supplements could improve hematological status of adolescent girls in low socio-economic group.

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