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Original Research Article

To Study the Oxidative Stress & It's Correlation with Vit B12 & Folic Acid Levels in Megaloblastic Anemia

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Conflict of interest: Nil

Abstract:

Introduction: WHO defines, megaloblastic anemia as a type of anemia characterized by the formation of unusually large, abnormal and immature red blood cells. Prevalence of megaloblastic anemia in India according to many studies ranges from 02%-40%.It has been found to have decrease antioxidant activity in anemic patient causing increase oxidative stress, leading to DNA damage and altered biomolecules.

Aim: To study the oxidative stress & it's correlation with vitamin B12 & folic acid levels in Megaloblastic anemia.

Methods: A Case Control Study was carried out from January 2023 to June 2023 by Medicine & Biochemistry department of Government Medical College, Datia(Madhya Pradesh) .100 non-pregnant female(age ranges 15-45yrs) patient having Hb<12g/dl & Megaloblastic anemia were taken as cases. Whereas 100, age and sex matched without anemia as controls. Blood sample was collected in EDTA vial for complete blood count & peripheral blood smear. Blood sample was collected in plain vial to estimate serum vitamin B12, folate & FRAP (Ferric Reducing Ability of Plasma). Serum B12 & folate estimation was done by Chemiluminiscence method & Spectrophotometric analysis was done for FRAP.

Results: Serum Vitamin B12 was found to be lower among 56% of cases (113.2±189.6) compared to controls (206.7±261.9). Serum Folate was found to be lower among 25% of cases (5.7±4.1) compared to controls (7.3±4.2).Both vitamin B 12 & folic acid, found to low among 19% of cases. Serum FRAP value was found to be lower among Cases (582.7±198) compared to controls (908.1±174.2).There was a positive significant (p-value<0.05) & mild correlation of Serum FRAP value with serum Vitamin B12 and Serum Folate in Controls. In Cases the correlation was stronger with positive significance (p-value<0.05), compared to Controls.

Conclusion: In our study, it may be concluded that in Megaloblastic anaemia there is serum folate and Vitamin B12 deficiency with increased oxidative stress leading to free radical generation and peroxidation of vital body molecules. Therefore in such cases, thorough biochemical analysis should be undertaken before starting with medications and there should be an antioxidant supplementation to be given.

Keywords: EDTA, FRAP, CBC.

Introduction

Megaloblastic anemia is defined as type of anemia characterized by the formation of unusually large, abnormal and immature red blood cells called as megaloblasts by the bone marrow, which are released into the blood [1]. Prevalence of Megaloblastic anemia in India according to many studies ranges from 02%-40% [2]. Various Nutritional Factors influence effective erythropoiesis and lead to impairment in RBC morphology. [1] The root cause of megaloblastic anemia in most of the cases is nutritional deficiencies of either Vitamin B12 or Folate deficiency due to socio-culture of our country. Peoples are strict vegetarians with the non- balanced diet, malabsorption or lack of intrinsic factor is the main causes of megaloblastic anemia, additionally vitamin B12 deficiency can potentially cause irreversible neurologic damage to the developing brain [3]. It has found in studies that decreased SOD (superoxide dismutase), glutathione peroxidase and catalase activity in anemia has been linked to increased oxidative stress, because it is well known that reactive oxygen species, specially hydrogen peroxide (H_2O_2), inhibits SOD activity & it can lead to oxidative stress [4], that oxidative stress causes DNA damage, impaired synthesis of protein,

membrane lipids and carbohydrates and altered cell proliferation[5-6]. Hence the present study was planned with an aim to estimate the levels Serum Vitamin B12, Serum Folate in megaloblastic anemia patients and their correlation with oxidative stress.

Material & Methods

Study Design: Case Control Study

A Case Control Study was carried out from January 2023 to June 2023 by Medicine & Biochemistry department of Government Medical College, Datia(Madhya Pradesh).100 non-pregnant females (age ranges 15-45yrs) patient having Hb<12g/dl & Megaloblastic anemic were taken as cases. Whereas 100 age and sex matched non-anemic females as controls.

Exclusion Criteria

Subjects who had undergone any major surgery in the last one month, who had history of acute blood loss in the last one month. Subjects who received blood transfusion in the last one month. Subjects with history of chemotherapy or radiation therapy. Subjects who were consuming iron or vitamin supplements. Subjects who were known cases of blood dyscrasias, Pregnant females, Smoker and alcoholic. Subjects having history of metabolic diseases such as diabetes mellitus, malignancy, heart diseases, or having infections such as tuberculosis, HIV, endocrine disorders.

Sample Collection and Processing

2ml of blood sample was collected in EDTA vial for complete blood count & peripheral blood smear. To estimate serum vitamin B12,folate & FRAP(Ferric Reducing Ability of Plasma) 4ml of blood sample was collected in plain vial.

Blood sample of confirmed Megaloblastic anemic cases through CBC & peripheral blood smear were analyzed to estimate serum B12 & folate by Chemiluminiscence & FRAP analysis was done via Spectrophotometer.

Ethical Considerations: The study was performed after obtaining approval from the Institutional Ethics Committee. A written informed consent was obtained from all the subjects. The participation in the study was entirely voluntary and the patients had complete autonomy and freedom to withdraw from the study whenever they wished to do so.

Results

Table 1: Overall status of serum biochemical parameters in Control group and Cases

		Serum Vit B12(187 883) pg/ml	Serum Folate (3.2-20.5 ng/ml)	Serum FRAP Value(612-1634µmol/Lt)
Controls (n=100)	Min	183	3.8	612
	Max	846	19.8	1386
	Mean ± SD	206.7±261.9	7.3±4.2	908.1±174.2
	SE	26.19	0.42	17.42
Cases (n=100)	Min.	66	0.6	267
	Max	228	19	1089
	Mean ± SD	113.2±189.6	5.7±4.1	582.7±198
	SE	18.96	0.41	19.8
p value (of cases and controls)		0.121	0.201	0.271

SD-Standard Deviation, SE-Standard Error. Above table shows that Serum Vitamin B12 was found to be lower among cases (113.2±189.6) compared to controls (206.7±261.9).

Serum Folate was found to be lower among cases (5.7±4.1) compared to controls (7.3±4.2). Serum FRAP value was found to be lower among Cases (582.7±198) compared to controls (908.1±174.2). There was a positive significant (p-value<0.05) mild correlation of Serum FRAP value with serum Vitamin B12 and Serum Folate in Controls & in Cases the correlation was stronger with positive significance (p-value<0.05), compared to Controls.

Table 2: Comparative Percentage distribution of Serum biochemical parameters in Cases (n=100) and Controls (n=100)

Controls (n=100)	Percentage population	SerumVit B12	Serum Folate	Both vitamin
	Less than normal Serum levels	0.0	0.0	0.0
	Within normal range	100	100.0	100.0
Cases (n=100)	Less than normal Serum levels	56	25	19
	Within normal range	44	75	81
p value (of cases and controls)		0.0004	0.0002	0.0001

Above tables shows significant change in percentage distribution of serum biochemical parameters in Controls and Cases. 25% of cases showed deficiency of Serum Folate levels, 56% cases showed deficiency of Serum Vitamin B12 & 19% cases showed deficiency of both vitamins. No of subjects with Serum Vitamin B12 deficiency also increased significantly (p=0.0004) in cases.

No of subjects with Serum Folate deficiency were found to be significantly (p=0.0002) higher in Cases, compared to Controls.

No of subjects with both vitamins were found to be significantly (p=0.0001) higher in Cases, compared to Controls.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software Version 24.

All quantitative data was expressed as mean + standard deviation. Qualitative variables were expressed as proportions or percentages. Comparison of categorical variables was made between groups using Chi – square test or Fischer's exact test as applicable.

Correlation between variables was assessed by calculating the Spearman's correlation coefficient. P value < 0.05 was considered statistically significant.

Table 3: Correlation between Serum Oxidative Stress indicator (FRAP) with other biochemical Parameters

Variables	FRAP(r)	p-value
Hb	0.683	0.0001
Serum Vit B12	0.478	0.001
Serum Folate	0.539	0.001

(r- relative of coefficient). In above table we found that there is a strong positive correlation which is statistically significant between Hb and Serum FRAP value among Megaloblastic anemic subjects (r= 0.683, p-value=0.0001). We have also found a positive correlation which is statistically significant between Serum Vitamin B12 and Serum FRAP value among cases (r= 0.478, p-value=0.001). We have also found a positive correlation which is statistically significant between Serum Folate and Serum FRAP value among cases (r= 0.539, p-value=0.001).

Table 5:

Groups	Correlation coefficient	p-value
Controls	0.263	0.008*
Cases	0.683	0.0001*

Spearman's correlation coefficient, *Significant. Above table shows the mild positive significant (p-value<0.05) correlation between Hb and Serum Oxidative Stress Indicator (FRAP Value) in Controls. In Cases the correlation was stronger with positive significance (p-value<0.05), compared to Controls.

Discussion

The root cause of megaloblastic anemia in most of the cases is nutritional deficiencies of either Vitamin B12 or Folate. Their deficiency results in various abnormalities because of their specific biochemical roles.

Although relationship of levels of various biochemical parameters has been studied in different independent studies, however there is lack of studies evaluating these relationships together in a single study. Hence the present study was planned with an aim to estimate the levels of Serum Vitamin B12, Serum Folate in megaloblastic anemic patients and controls and to establish correlation of these biochemical parameters with Serum Antioxidant levels. Numerous studies have been independently conducted that Antioxidant activity is decreased in anemia are Acharya et al [7], Bartal M et al [8], Erdal Kurtoglu et al [9], Mehmat Aslan et al [10]. Our study also shows that Antioxidant potential of Controls (Mean value of FRAP =206.7), is higher than that of Cases (Mean value of FRAP =113.2).

Contrary to our results Al-Abrash AS et al [11], found that Catalase activity is increased in Anemia. Whereas our study states that Antioxidant activity is decreased in Megaloblastic Anemia.

Limitation

Serum MMA (Methyl Malonic Acid) & Holotranscobalamin estimation are more sensitive and specific marker for vitamin B12 deficiency.

Conclusion

There was a positive significant (p-value<0.05) mild correlation of Serum FRAP value with serum Vitamin B12 and Serum Folate in Controls. In Cases the correlation was stronger with positive significance (p-value<0.05), compared to Controls. There is mild positive significant (p-value<0.05) correlation between Hb and Serum Oxidative Stress

Indicator (FRAP Value) in Controls. In Cases the correlation was stronger with positive significance (p-value<0.05), compared to Controls. We found that Vitamin B12 & folic acid both is low among cases & controls. In our study, it may be concluded that in megaloblastic anemia there is serum folate and Vitamin B12 is deficient with increase oxidative stress and free radical generation and peroxidation of vital body molecules. Therefore in such cases, thorough biochemical analysis should be undertaken before starting with medications and there should be an antioxidant supplementation is given.

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