IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 15, Issue 6 Ver. XIII (June. 2016), PP 01-05
www.iosrjournals.org

The Study of Relationship of Alpha Fetoprotein and CA-125 Levels with Iron Overload in the □-Thalassemic Tribal Children Receiving Multiple Blood Transfusions

Suman Chatterjee[1], Santa Saha-Roy[2*], Sumanta Banerjee[3], Dibakar Haldar[4], Pinaki Sarkar[5], Tanima Mandal[6].

[1](P.G.T., Department Of Biochemistry, B.S.M.C., Bankura, India)
[2] (Associate Professor, Department Of Biochemistry, B.S.M.C., Bankura, India)
[3] (P.G.T., Department Of Biochemistry, B.S.M.C., Bankura, India)
[4] (Associate Professor, Department Of Community Medicine, B.S.M.C., Bankura, India)
[5] (Professor, Department Of Biochemistry, B.S.M.C., Bankura, India)
[6] (Demonstrator, Department Of Biochemistry, B.S.M.C., Bankura, India)
[*] Corresponding author

Abstract: *Blood transfusion is the mainstay of treatment for Beta thalassemia, an inherited blood disorder and a major public health problem. Such treatment leads to extensive iron-induced injury in the heart, liver, pancreas and endocrine system. Paediatric tribal population receiving multiple blood transfusions for treatment of β-thalassemia has been studied to evaluate the relationship between iron overload with AFP and CA125 and to predict early liver damage. Information pertaining to demographics, blood transfusion and the nature of chelation therapy etc. was obtained on thirty-two patients, attending Bankura Sammilani Medical College, Bankura, and receiving multiple blood transfusions. Blood samples were analyzed for assessment of serum Ferritin, AFP and CA125 concentrations by ELISA and compared with age and sex matched thirty-three healthy controls. The serum ferritin was found to have a statistically significant positive linear relationship with age and AFP in the study group whereas a significant positive linear relationship between AFP and CA125 existed in the control group. Serum AFP was higher in the study group compared to that of the comparison group. Serum AFP level can be used as a potential early predictor of ongoing liver damage among the thalassemic patients with elevated levels of ferritin receiving multiple blood transfusion.*

Keywords: *AFP, β-thalassemia major, CA-125, early liver damage, serum ferritin,*

I. Introduction

Thalassemia, the autosomal recessive syndrome is one of the most common monogenic disorders in the world[1]. It arises from mutation in globin gene (s) leading to a reduction or absence of haemoglobin, that can range from insignificant to life threatening[2]. β-thalassemia is the commonest single-gene disorder in the Indian population[3]. Haemoglobinopathies are the commonest hereditary disorders in India and pose a major health problem. Ten percent of the total world thalassemics are born in India every year[4]. The data on the prevalence of β-thalassemias and other haemoglobinopathies in different caste or ethnic groups of India is scarce[5]. Certain communities in India like Sindhis, Gujratis, Punjabis and Bengalis are more commonly affected with β thalassemia, the incidence varying from 1 to 17%[6]. From the reports of previous studies it is found that thalassemia and other haemoglobinopathies are prevalent among the tribal communities of India and make a public health problem among them[7]. Haemoglobinopathies particularly those related to haemoglobin S and E (HbS, HbE) and β-thalassaemia are important challenges for tribal populations in India constituting approximately 8.5 per cent of the total population [8,9]. The tribal population is high in Bankura district of West Bengal. A study carried out by Chakrabarti et al in Bankura district on both tribal and non-tribal antenatal mothers showed that the prevalence of haemoglobinopathies were more common in tribal communities[10].

The mainstay of treatment in β-thalassemia major is blood transfusion to maintain adequate level of hemoglobin. Iron overload in β-thalassemia major patients is secondary to multiple blood transfusions and increased iron absorption[11]. In patients receiving multiple blood transfusions, excessive iron potentially catalyzes generation of free-radicals and impairment in cellular function and integrity due to imbalance of the redox system[12]. Extensive iron-induced injury develops in the heart, liver, pancreas and endocrine system[13]. The concentration of serum ferritin gives a quantitative measure of the amount of storage iron in normal subjects and those with iron deficiency or overload of any cause [14].

Alpha fetoprotein (AFP) is synthesized in large quantities during embryonic development by the fetal yolk sac and liver. In addition to pregnancy, elevated concentrations of serum AFP are associated with benign

liver conditions, such as hepatitis and cirrhosis. AFP is also a marker for hepatocellular and germ cell (nonseminoma) carcinoma[15].
CA-125 level is an well known tumour marker of ovarian cancer but many other benign and malignant conditions such as pregnancy, cardiovascular and liver diseases can give rise to elevated CA-125 levels[15].
In this background, this study has been conducted with an aim to evaluate the relationship between iron overload with AFP and CA125 and to predict early liver damage in β-thalassemia major in tribal children receiving multiple blood transfusions.

II. Materials And Methods

This descriptive cross-sectional study was carried out during the period from November 2015 to January 2016 in the Department of Biochemistry, Bankura Sammilani Medical College (B.S.M.C.), Bankura.
Thirty-two patients admitted in the thalassemia daycare unit at in-patient department of Paediatric Medicine, B.S.M.C., Bankura were included in the study group.

2.1. Inclusion criteria:

Children suffering from β-thalassaemia major from tribal population of Bankura district, and receiving at least 3 times blood transfusion in Bankura Sammilani Medical College and Hospital. However the last blood transfusion was taken at least one month prior to blood collection.

2.2. Exclusion criteria:

1) Diabetes Mellitus, 2) Existing renal failure (serum creatinine >3 mg/dl), 3) Any existing haematological or other malignancy, 4) Severe anemia (Hb <6g/dl), 5) Existing liver diseases, Hepatitis B, Hepatitis C and 6) Known active infections.
Both age and sex matched 33 healthy children were selected as control.
Necessary permission from Institutional ethical committee had been taken.

2.3. Laboratory investigations:

The blood samples were collected from the patients and sera were separated by centrifuge and stored at -20ºC before analyzing. Serum AFP, CA-125 and ferritin levels were estimated by immunoassay using ELISA reader.

2.4. Statistical analysis:

The data were compiled in MS excel and analyzed by different statistical methods. Data display was done by charts and tables. Data were described by proportion, mean, SD, range etc. Statistical tests like independent 't' test, Chi-square test, Odds ratio (OR) with 95% confidence interval (CI), Spearman correlation coefficient (r), multiple linear regression analysis etc. were used to explore the relationship between variables. P value of < 0.05 was considered significant to discard the null hypothesis at 5% precision and 95% confidence interval. Statistical package for Social Sciences (SPSS), version 22 was utilised for the purpose of analysis.

III. Results

Out of 65 participants 35.38% were female. The groups were not different in respect of gender distribution [$\chi 2=0.08$, $p=0.867$ at $df=1$; OR=1.09(0.39-3.02)] (Fig.1).

The average age of the participants was 6.92±2.94 (mean ± SD) years with a range of 11 years. It was revealed that the two groups were comparable with regards to the age of their members having statistically significant difference (Table-1). However, the study group was found to have higher average serum ferritin, AFP and CA125 levels compared to that of the comparison group and the difference was statistically robust (Table-1).

The serum ferritin was found to have a statistically significant positive linear relationship with age and AFP among the subjects belonged to the study group whereas analysis revealed a significant positive linear relation between AFP and CA125 among the subjects of comparison group (Table-2).

Multivariate analysis between serum ferritin concentration and age of the patients, serum AFP and CA 125 levels showed that age had a significant impact on the rise of serum ferritin level in the study group only. It was evident that with the increase of age by 1 year the serum ferritin was found to increase by 36.532 units (Table-3). No other serum marker e.g. AFP and or CA125 was found to have any correlates of increase in their serum concentration. It might be due to the higher cumulative blood transfusion received by the thalassemic subject with increasing age as well as poor chelation therapy. The positive linear relation between the AFP and CA125 in comparison group had showed no significant model fit and hence might be spurious (Table-2).

IV. Discussion

AFP, a glycoprotein, is one of the major proteins in the fetal circulation, but its maximum concentration is about 10% that of albumin. The serum AFP concentration is less than 10µg/L in healthy individual. During pregnancy, maternal AFP concentration increases from 12 weeks' gestation to third trimester. In addition to pregnancy, elevated concentration of serum AFP is associated with benign liver conditions, such as hepatitis and cirrhosis; AFP is also useful in monitoring of therapy for hepatocellular carcinoma [15]. A study conducted by Yu-rui Liu showed clinical significance of serum AFP levels in diagnosing liver inflammation and fibrosis [16]. In our study the study group was found to have higher serum AFP compared to that of the comparison group (Table-1).

CA-125 is a high molecular mass glycoprotein recognized by the monoclonal antibody OC 125. In a healthy individual, the upper limit of CA-125 is 35 kU/L[15]. A study conducted by Yeganeh-Amirkande S et al. showed that significant increase in tumor marker CA125 in patients with hepatitis C[17]. CA-125 level is not only elevated in ovarian cancer but many malignant and benign conditions such as pregnancy, cardiovascular and liver diseases can give rise to elevated CA-125 levels. Elevation of CA-125 levels has been reported in patients with liver disease and its elevation is common in patients with liver cirrhosis a study conducted by Quresh Omar M showed that was a moderate correlation between CA-125 levels and amount of ascites[18]. In this study, the thalassemic patients were found to have higher CA-125 levels compared to that of the control group (Table-1).
Multiple blood transfusions in thalassemic patients lead to iron overload. Three measures of iron overload were studied: (i) chemical iron content in the initial liver biopsy specimen, (ii) mean serum ferritin during follow-up and (iii) the trend in serum ferritin during follow-up expressed as a risk grading[19]. In our study a significant higher concentration of serum ferritin was found in study group compared to controls (Table-1).

Because of ineffective erythropoesis thalassemic patients have high rate of iron absorption from the gut. They suffer from high serum iron both resulted from high absorption and increased turn over of RBC[20]. Excess iron gets deposited in liver, heart, pancreas, kidney, suprarenals, gastrointestinal tract etc.[21] and responsible for various adverse events among thalassemics unless it is taken care of. So clearance of this high serum iron concentration is one of the mainstays of treatment of thalassemics and it is done by chelation therapy[22]. Monitoring of effective chelation therapy is helpful for prevention of this harmful consequence arising out of iron overload. Inadequate chelation therapy or absence of it, results in early stage of potential benign liver disease or damage (cirrhotic change) reflected in rise of AFP and CA125 level in serum in comparison to the control group. The rising AFP level in the study group may be due to benign condition like cirrhosis or haematochromosis etc.

The condition is also indicated by elevated ferritin concentration reflecting iron overload in the subjects with beta-thalassemia receiving multiple blood transfusions[14]. Iron is toxic to many vital organs and the injury is often mediated by iron induced oxidative stress[23]. The toxic effects of iron deposition are related with number of blood transfusions which depends on the age of the patient also. It has an inverse relation with the successful chelation of iron. In this study, both AFP and CA-125 were evaluated as markers of possible organ involvement. The levels of both were elevated in the study group in spite of being within the normal range in comparison to the control group. The elevation of serum AFP had shown significant correlation with serum ferritin levels and hence iron overload.

V. Conclusion

From the findings of the present study a rise in serum AFP level can be a used as potential early predictor of ongoing liver damage among thalassemics with elevated levels of ferritin receiving multiple blood transfusions. Further validation of the present findings with evaluation of other markers of liver injury and histopathological findings is necessary. Thus routine measurement of the serum markers of haemochromatosis is essential for monitoring effective chelation therapy which prolongs the life span of thalassemics and can prevent consequences of iron overload.

References

[1]. Gamberini MR, De Sanctis V, Gilli G. Hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism: Incidence and prevalence related to iron overload and chelation therapy in patients with thalassaemia major. Pediatr Endocrinol Rev. 2008;6:158–69.
[2]. Thein SL. Genetic modifiers of thalasemia. Hematologica.2005; 90: 649-60.
[3]. Verma IC. The challenge of genetic disorders in India. In: Molecular genetics and gene therapy- the new frontier.Scientific Communications, Amsterdam.1994; 1-20.
[4]. Bashyam MD, Bashyam L, Savithri GR. et al.Molecular genetic analyses of beta thalassemia in South India reveal rare mutations in the beta globin gene. J Hum Genet. 2004; 49: 408-13.

[5]. Mohanty D , Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, Sharma SK, Chaudhari U,Ghosh M, Das S, Britt RP, Singh S, Ross C, Jagannathan L, Kaul R, Shukla DK, Muthuswamy. Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: a multicentre study.J Community Genet.Jan.2013;4(1): 3342.
[6]. Gupta A, Hattori Y, Gupta UR et al. Molecular genetic testing of beta thalassemia patients of Indian origin and a novel 8bp deletion mutation at codons 36/37/38/39. Genet Test. 2003; 7: 163-8.
[7]. Sengupta M.Thalassemia among the tribal communities of India. The Internet Journal of Biological Anthropology. 2008; Volume1 .Number 2.
[8]. Ghosh et al. Haemoglobinopathies in tribals. Indian J Med Res .2015; 141:505-8.
[9]. Census of India 2011, Office of the Registrar General and Census Commissioner. Ministry of Home Affairs, Govt. of India. Available from: http;// www.censusindia.gov.in.
[10]. Chakrabarti S, Mandal K, Pathak S, Patra A, Pal S. Haemoglobinopathies among the tribal and non-tribal antenatal mothers in a tertiary care hospital of rural West Bengal, India. Bangladesh Journal of Medical Science. 2016; Vol.15 No.01:90-4.
[11]. Toumba M, Sergis A, Kanaris C, Skordis N. Endocrine complications in patients with Thalassaemia Major. Pediatr Endocrinol Rev. 2007; 5: 642–8.
[12]. Hasan NA, Musa RJ, Abdullah SA. Effect of Iron Overload and Lipid Peroxidation on Hyroid Function in Iraqi Patients with ß Thalassemia. Asian Journal of Applied Sciences. 2013; Vol 1, No 5.
[13]. Prabhu R et al. Iron Overload In Beta Thalassemia - A Review. J Biosci Tech.2009; Vol 1 (1):20-31.
[14]. Jacobs A, miller F, Worwood M, Beamish MR, Wardrop CA. Ferritin in the Serum of Normal Subjects and Patients with Iron Deficiency and Iron Overload. British Medical Journal.1972; 4:206-8.
[15]. Burtis CA, Burns DE. Tietz Textbook of Clinical Chemistry and Molecular Diagnostic. Elsevier; 2012.
[16]. Liu et al. Alpha-fetoprotein level as a biomarker of liver fibrosis status: a cross-sectional study of 619 consecutive patients with chronic hepatitis B. BMC Gastroenterology .2014;14:145.
[17]. Amirkande SE et al. The frequency of CA15-3, CA125, CA19-9 in Patients with Hepatitis B and C .Zahedan Journal of Research in Medical Sciences Journal.2015.
[18]. Qureshi MO, Dar FS, Khokhar N. CancerAntigen-125 as a Marker of Ascites in Patients with Liver Cirrhosis. Journal of the College of Physicians and Surgeons .2014; Vol. 24 (4): 232-35.
[19]. Telfer PT, Prestcott E, Holden S,Walker M, Hoffbrand V and Wonke B. Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassaemia major. British Journal of Haematology. 2000;Volume 110, Issue 4: 971–977.
[20]. Milena R, Branka Z, Biljana S et al. Thalassemia syndrome in Serbia. Heamoglobin 2010; 34(5):477-85.
[21]. Gupta et al. Production Of Reactive Oxygen Species, Its Effect, Drugs And Plant Extract Used As An Antioxidant, Chelator On Thalassemic Patient: A Review. IJPSR.2011; Vol. 2(9): 2278-85.
[22]. Olivieri NF ,Brittenham GM. Iron-Chelating Therapy and the Treatment of Thalassemia. Blood Journal. 1997;89 (3).
[23]. Bhagat SS, Dey Sarkar P, Suryakar AN, Padalkar RK, Karnik AC, Ghone RA et al. A Study on the Biomarkers of Oxidative Stress: the Effects of Oral Therapeutic Supplementation on the Iron Concentration and the Product of Lipid Peroxidation in Beta Thalassemia Major.journal of clinical and diagnostic research.2012; Vol 6:.1144-47.

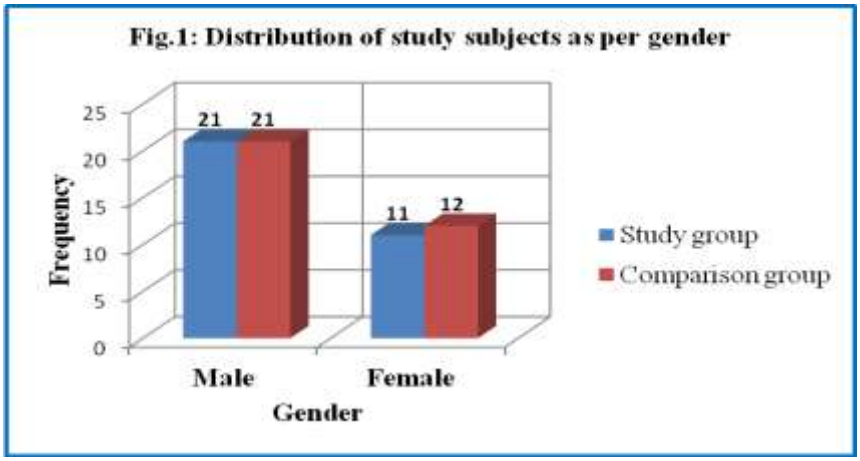

Fig. 1: Distribution of study subjects as per gender

Table-1: Distribution of groups according to different parameters

Parameter	Group	Mean ± SD	Unpaired 't' test, p*
Age	Study	7.11±3.11	0.496, 0.622
	Comparison	6.75±2.0	
Serum ferritin	Study	655.34±245.18	**13.224, 0.000**
	Comparison	84.69±36.37	
AFP	Study	4.94±3.15	**7.579, 0.000**
	Comparison	0.77±0.33	
CA125	Study	15.76±16.81	**3.206, 0.002**
	Comparison	4.97±9.41	

Table-2: Relationship between the age and the serum parameters among two groups

Parameter	Group	Correlation	Significance

		coefficient (r)	**(p value)**
Age Vs serum ferritin	Study	0.452	**0.005**
	Comparison	0.204	0.128
Age Vs AFP	Study	0.125	0.247
	Comparison	0.106	0.279
Age Vs CA125	Study	0.205	0.130
	Comparison	0.125	0.244
Serum ferritin Vs AFP	Study	0.302	**0.046**
	Comparison	0.196	0.137
Serum ferritin Vs CA125	Study	0.010	0.478
	Comparison	0.060	0.371
AFP Vs CA125	Study	0.218	0.116
	Comparison	0.394	**0.012**

Table-3A: Model Summary of multivariate analysis

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.552[a]	.304	.201	219.13969	.304	2.952	4	27	.038

a. Predictors: (Constant), Ca125group2, Agegroup2, AFPgroup2, Gender2.

Table-3B: ANOVA[a] for estimating model fit in multivariate analysis in study group

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	566951.104	4	141737.776	2.952	.038[b]
	Residual	1296599.547	27	48022.205		
	Total	1863550.651	31			

a. Dependent Variable: Ferritingroup2

b. Predictors: (Constant), CA125group2, Agegroup2, AFPgroup2, Gender2

Table-3C: Coefficients[a] derived from linear regression analysis in study group

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	372.881	124.350		2.999	.006	117.735	628.027
	Gender2	-61.485	84.734	-.121	-.726	.474	-235.344	112.375
	Agegroup2	36.532	13.058	.463	2.798	.009	9.739	63.326
	AFPgroup2	21.944	12.853	.282	1.707	.099	-4.429	48.317
	CA125group 2	-2.880	2.529	-.197	-1.138	.265	-8.069	2.310

a. Dependent Variable: Ferritin group2