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TO STUDY THE HAEMOGLOBINOPATHIES AND RATIO OF COPPER AND ZINC IN SINDHI COMMUNITY OF BHOPAL

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ABSTRACT

The genetic haemtological disorder such as beta–thalassaemia and sickle cell anaemia is one of the burning problems in India. The community wise study is of great help as it provides versatile information. A random cross-sectional study was conducted in 500 volunteers of Sindhi community belonging to all age groups and both sexes. The mass screening was done by help of NESTROFT, Sickling, Solubility and complete blood picture were performed on all samples along with BMI .Those positive for either one or both, screening tests were further analyzed for HbA2 by HPLC D-10. 120 positive cases of haemoglobinopathies were further analyzed for copper /zinc ratio. 120 positive cases of haemoglobinopathies were having low level of zinc and high level of copper, the ratio of copper / zinc was high along with low BMI. The positive cases were having different clinical manifestations along with liver and heart diseases in some cases.

KEYWORDS: Beta–Thalassaemia, Sickle Cell Anaemia, NESTROFT, Sickling, Solubility, Copper /Zinc Ratio

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INTRODUCTION

Haemoglobinopathies are genetic disorder of the structure and function of haemoglobin and are of great public health concern[1]. Haemoglobinopathies include structural variants of haemoglobin such as sickle haemoglobin (HbS) and disorders in which the synthesis of one or more globin molecules is reduced or absent (the thalassaemias) [2-4]. The term thalassaemia is derived from the Greek, thalassa (sea) and haima (blood) [5]. It is prevalent across the world due to migration [6]. Alpha-thalassaemia is a common hereditary condition caused by deletions or point mutations in one or both alpha-globin genes, located on chromosome 16 [7-8]. Beta –thalassaemia is caused by more than 200 point mutations in the ß- globin genes on chromosome 11[9] resulting in absent or decreased synthesis of β- globin chains causing imbalance between α- and β-chains and ensuing ineffective erythropoeisis, hemolysis and anaemia [10-12]. Sickle cell anemia results from point mutation in one of the amino acids forming globin chain of hemoglobin by the substitution of a hydrophilic moiety (Glutamic acid), negatively charged by a hydrophobic amino acid (Valine), and cause neutral charge at the sixth position of β-chain of hemoglobin molecule. Unlike normal haemoglobin sickle haemoglobin has net positive charge which results in reduce solubility. Upon deoxygenation in microcirculation sickle haemoglobin molecule reversibly aggregates into paracrystalline polymers, which forms long fiber bundle and distort erythrocytes giving characteristics distorted sickled shapes RBC's [13-17].

Trace Elements

Trace elements play a pivotal role in the human body and participate in various bio-chemical reactions. Zinc is the abundant intracellular element with 85% of total zinc found in muscles and bone where as 0.1 % in the plasma [18]. It forms structural part of more than 300 metalloenzymes like super oxide dismutase [19]. Plays an essential role in human growth, development [20] acts as an antioxidant, [21-22] synthesis, storage and secretion of insulin,[23-24] host defense mechanisms[25-28]and in thyroid metabolism, its deficiency causes reduction in concentration of T3 in plasma [29-30]. Impaired growth, alopecia, loss of weight are few complications due to zinc deficiency, which is one of the factors responsible for growth and puberty disorders in thalassemic patients [31]. Copper is an essential structural element of many enzymes acting as cofactor in enzymatic reactions like those of cytochrome C, Lysyl oxidase, superoxide dismutase [32-33]. It helps in growth and proliferation of healthy cells, lymphocytes maturation and regulates the immune function[34]. It maintains the elasticity of the skin, blood vessels, and lungs and has antioxidant, antibacterial property.

MATERIALS AND METHODS

The study was conducted to screen the haemoglobinopathies in Sindhi community living in Bhopal, Madhya Pradesh (MP). In this prospective cross sectional study 500 subjects (300 males and 200 females) between age group from 1 to 50 years were examined.

i. Physical Examination

The family history, related complications of diseases, physical examination, height and weight were ascertained by physician.

ii. Sample Collection

5 ml of venous blood was drawn from the cubital vein using disposable needle and syringes after having written consent of the patients. Blood sample was divided into two parts (1) one part in EDTA vial for hematological and HbA2 analysis (2) one in plain vial for collecting the serum.

iii. NESTROFT[35-36]

The mass screening was done with the help of NESTROFT. 2 ml of 0.36% buffered saline solution and 2 ml distilled water was taken in tube. A drop of blood was added to each tube and left undisturbed for 1/2 an hour at room temperature. The samples were examined using light against the background of black

lines. The results were interpreted as positive when the black lines were not visible. A positive test indicates lowered red cell osmotic fragility, suggestive of thalassemia trait.

iv. Sickling test [37]

Twenty micro liters of each blood sample, was mixed with 20 micro liters of 2% sodium metabisulphite on a cover slip. A slide was then gently pressed onto the cover slip and the cover-slip was ringed with candle wax. The slide preparations were left in a humidified chamber for 15 minutes at room temperature and then examined under the microscope (x10). Further observations were taken after 30 minutes, 1 and 2 hours interval. Sickling was considered to be positive when more than 25% of the red blood cells were sickled.

v. Solubility Test [37]

Twenty micro-liters of each sample were mixed with 2 ml of 0.02% sodium dithionate in a test tube and left to stand at room temperature for 5 minutes. The samples were examined using light against the background of black lines. The results were interpreted as positive when the black lines were not visible.

vi. Blood Analysis [38]***:***

The complete blood picture was analyzed by Automated Haematology Analyzer. The EDTA blood samples were used.

vii. HbElectrophoresis [39]***:***

The NESTROFT and Solubility positive samples were analyzed for haemoglobinipathies (thalassaemia & sickle cell disease) by help of HPLC D-10.

viii.Copper / Zinc measurement [40-41]

The level of copper and zinc were assessed in subjects having haemoglobinopathies with the help of atomic absorption spectrophotometer (SHIMADZU Model AA-6300), used in accordance with the manufacturer's operating manual.

FINDINGS

According to the study 140 out of 500 subjects were NESTROFT & solubility test positive. 140 positive subjects were taken for further study. The table 1 and 2 depicts the breakup of sample according to the gender and diagnosis of various haemoglobinopathies.

Table No. 1
Showing the distribution of the samples according to the gender

Variables	Number	Percentage	Cumulative Percentage
Male	88	73.33	73.33
Female	32	26.67	100

Table No. 2
Depicting the distribution of the samples according to the diagnosis in 120 subjects

Variables	Number of subjects	Percentage	Cumulative Percentage
ß-thalassaemia minor	77	64.16	64.16
ß-thalassaemia major	14	11.66	75.82
Sickle beta-thalassaemia(Hb S/β Th)	16	13.33	89.15
Sickle cell trait (hgb AS)	9	7.50	96.65
Sickle cell Disease (SCD)	3	2.50	99.15
Hemoglobin E (HbE)	1	.85	100

The table 3 representing the values of chromatogram and haematogram of all the 120 subjects having different haemoglobinopathies. The figure No.1 represents the haematograms and chromatogram of the patients having haemoglobinopathies.

Table No. 3

Depicting the Values of Chromatogram & Haematogram Readings of Subject of Haemoglobinopathies.

No.Subject	A_O	F	A_2	S.W.	RBC	Hb	MCV	MCH	MCHC	RDW	Findings
T-1	82.6	>1	7.7	-	6.69	13.8	68.6	20.7	27.1	18.2	ß-thalassaemia minor
T-2	83.6	>1	5.6	-	6.06	11.9	65.1	19.6	30.1	17.4	ß-thalassaemia minor
T-3	79.2	>1	6.3	2.0	5.0	10.2	57.1	24.7	25.2	16.02	ß-thalassaemia minor
T-4	86.2	>1	4.2	-	4.5	9.1	61.0	28.4	19.02	14.3	ß-thalassaemia minor
T-5	65.8	>1	6.3	-	4.17	9.2	66.7	22.1	33.1	26.2	ß-thalassaemia minor
T-6	68.5	>1	5.8	-	3.9	8.0	65.4	18.8	31.0	32.4	ß-thalassaemia minor
T-7	79.7	>1	4.2	3.0	3.31	6.6	86.1	28.7	33.3	51.3	ß-thalassaemia minor
T-8	79.4	>1	9.9	-	5.44	10.2	61.8	18.8	30.4	36.2	ß-thalassaemia minor
T-9	84.8	>1	4.2	-	4.9	8.8	65.4	17.9	27.4	45.0	ß-thalassaemia minor
T-10	84.4	>1	5.4	-	5.33	16.3	88.0	30.6	34.8	44.0	ß-thalassaemia minor
T-11	82.3	>1	5.9	-	4.72	7.4	57.2	15.7	27.4	41.7	ß-thalassaemia minor
T-12	83.5	>1	6.5	-	4.89	7.5	56.0	15.3	27.4	46.7	ß-thalassaemia minor
T-13	61.3	>1	3.7	2.0	4.50	6.9	58.0	15.3	26.4	41.4	ß-thalassaemia minor
T-14	10.0	>1	5.4	60.4	5.51	13.6	76.0	24.7	32.5	40.2	Hb S/β Th
T-15	60.0	>1	3.1	40	3.98	6.8	64.8	17.1	26.4	50.7	hgb AS
T-16	28.0	12	4.5	82	4.18	11.7	82.3	28.0	34.0	47.1	Hb S/β Th
T-17	81.2	>1	8.8	1.0	5.71	10.2	59.4	17.9	30.1	34.5	ß-thalassaemia minor
T-18	80.8	>1	5.4	2.0	5.37	9.6	60.5	17.9	29.5	35.9	ß-thalassaemia minor
T-19	11.0	9.0	5.9	52	6.59	11.5	59.2	17.5	29.5	37.5	Hb S/β Th
T-20	83.9	>1	4.0	2.4	4.69	7.7	61.4	16.4	26.7	39.4	ß-thalassaemia minor
T-21	62.4	>1	3.8	3.2	4.74	7.3	56.3	15.4	27.3	39.2	ß-thalassaemia minor
T-22	65.7	>1	4.2	1.0	5.47	9.8	60.1	17.9	29.8	36.6	ß-thalassaemia minor
T-23	80	5.0	3.7	89.0	4.71	12.4	82.8	26.3	31.8	42.9	Hb S/β Th
T-24	18.0	10	3.9	59	3.94	12.2	89.6	31.0	34.6	47.0	Hb S/β Th
T-25	81.7	>1	3.9	2.5	4.46	11.2	81.8	25.1	30.7	48.0	ß-thalassaemia minor
T-26	80.4	>1	8.1	3.0	5.70	9.4	58.2	16.5	28.3	37.2	ß-thalassaemia minor
T-27	85.1	>1	4.5	4.3	4.25	12.1	90.4	28.5	31.5	44.9	ß-thalassaemia minor
T-28	83.6	>1	5.6	-	6.06	11.9	65.1	19.6	30.1	17.4	ß-thalassaemia minor
T-29	79.2	>1	6.3	-	5.0	10.2	57.1	24.7	25.2	16.2	ß-thalassaemia

											minor
T-30	86.2	>1	4.2	-	4.5	9.01	61.0	28.4	19.02	14.3	ß-thalassaemia minor
T-31	82.3	>1	5.9	-	4.72	7.4	57.2	15.7	27.4	41.7	ß-thalassaemia minor
T-32	65.0	11	3.4	38	4.18	11.7	82.3	28.5	34.1	47.1	hgb AS
T-33	82.7	>1	5.9	-	4.61	7.0	57.4	15.3	28.4	40.5	ß-thalassaemia minor
T-34	82.3	>1	5.9	-	4.72	7.4	57.2	15.7	27.4	41.7	ß-thalassaemia minor
T-35	83.5	>1	6.5	-	4.89	7.5	56.0	15.3	27.4	46.7	ß-thalassaemia minor
T-36	54.0	21	3.3	39	4.71	12.4	82.8	26.3	31.8	42.9	hgb AS
T-37	0.0	21	3.8	92	3.94	12.2	89.6	31.0	34.6	47.0	Hb S/β Th
T-38	78	>1	3.2	2.3	4.69	3.2	61.4	16.4	26.7	39.4	ß-thalassaemia major
T-39	86.2	>1	4.4	-	4.5	9.1	61.0	28.0	19.02	14.3	ß-thalassaemia minor
T-40	65.8	>1	6.5	-	4.17	9.2	66.7	22.1	33.1	26.2	ß-thalassaemia minor
T-41	65.7	>1	3.6	1.0	5.47	9.8	60.1	17.9	29.8	36.6	ß-thalassaemia major
T-42	80.9	>1	3.1	-	3.98	6.8	64.8	17.1	26.4	50.7	ß-thalassaemia major
T-43	0.0	9	3.7	89	4.18	11.7	82.3	28.0	34.0	47.1	Hb S/β Th
T-44	51.6	>1	4.7	33.8	3.94	12.2	89.6	31.0	34.6	47.0	Hb S/β Th
T-45	85.1	>1	4.5	4.3	4.25	12.1	90.4	28.5	31.5	44.9	ß -thalassaemia minor
T-46	68.5	>1	5.8	-	3.9	8.0	65.4	18.8	31.0	32.4	ß- thalassaemia minor
T-47	62.4	>1	3.8	3.2	4.74	7.3	56.3	15.4	27.3	39.2	ß-thalassaemia major
T-48	58.4	>1	6.0	-	4.71	12.4	82.8	26.3	31.8	42.9	ß -thalassaemia minor
T-49	82.3	>1	5.9	-	4.72	7.4	57.2	15.7	27.4	41.7	ß -thalassaemia minor
T-50	84.2	>1	4.2	-	4.9	8.8	65.4	17.9	27.4	45.0	ß -thalassaemia minor
T-51	84.6	>1	5.4	-	5.33	16.3	88.0	30.6	34.8	44.0	ß -thalassaemia minor
T-52	82.3	>1	5.9	-	4.72	7.4	57.2	15.7	27.4	41.7	ß -thalassaemia minor
T-53	65.7	>1	4.2	1.0	5.47	9.8	60.1	17.9	29.8	36.6	ß -thalassaemia minor
T-54	0.0	22.0	4.7	83.0	4.71	12.4	82.8	26.3	31.8	42.9	Hb S/β Th
T-55	79.4	>1	9.9	-	5.44	10.2	61.8	18.8	30.4	36.2	ß -thalassaemia minor
T-56	79.4	>1	5.9	4.0	6.59	11.5	59.2	17.5	29.5	37.5	ß -thalassaemia minor
T-57	80.2	>1	5.5	-	4.78	8.5	56.3	16.4	25.3	40.5	ß -thalassaemia minor
T-58	82.3	>1	5.9	-	4.72	7.4	57.2	15.7	27.4	41.7	ß -thalassaemia minor
T-59	5.3	12.0	3.7	49.0	4.18	11.7	82.3	28.5	34.1	47.1	Hb S/β Th
T-60	56.4		3.1	30.7	4.71	12.4	82.8	26.3	31.8	42.9	hgb AS
T-61	61.3	>1	3.7	2.0	4.50	6.9	58.0	15.3	26.4	41.4	ß-thalassaemia major
T-62	60.1	21	3.4	39.0	5.51	13.6	76.0	24.7	32.5	40.2	hgb AS
T-63	80.9	>1	3.1	4.0	3.98	6.8	64.8	17.1	26.4	50.7	ß-thalassaemia

											major
T-64	64.0	7.7	3.7	89.0	4.18	11.7	82.3	28.0	34.0	47.1	Hb S/β Th
T-65	81.2	>1	8.8	1.0	5.71	10.2	59.4	17.9	30.1	34.5	ß -thalassaemia minor
T-66	80.8	>1	5.4	2.0	5.37	9.6	60.5	17.9	29.5	35.9	ß -thalassaemia minor
T-67	79.4	>1	5.9	4.0	6.59	11.5	59.2	17.5	29.5	37.5	ß -thalassaemia minor
T-68	83.9	>1	4.0	2.4	4.69	7.7	61.4	16.4	26.7	39.4	ß -thalassaemia minor
T-69	61.3	>1	3.7	2.0	4.50	6.9	58.0	15.3	26.4	41.4	ß-thalassaemia major
T-70	0.0	24	3.9	78.0.	5.51	13.6	76.0	24.7	32.5	40.2	Hb S/β Th
T-71	80.9	>1	3.1	4.0	3.98	6.8	64.8	17.1	26.4	50.7	ß-thalassaemia major
T-72	83.9	>1	4.0	2.3	4.69	7.7	61.4	16.4	26.7	39.4	ß -thalassaemia minor
T-73	86.2	>1	4.4	-	4.5	9.1	61.0	28.0	19.02	14.3	ß-thalassaemia minor
T-74	65.8	>1	6.5	-	4.17	9.2	66.7	22.1	33.1	26.2	ß -thalassaemia minor
T-75	65.7	>1	4.2	1.0	5.47	9.8	60.1	17.9	29.8	36.6	ß -thalassaemia minor
T-76	80.9	>1	3.1	4.0	3.98	6.8	64.8	17.1	26.4	50.7	ß-thalassaemia major
T-77	0.0	12	3.2	91.0	4.18	11.7	82.3	28.0	34.0	47.1	SCD
T-78	50.0	<1	3..2	37.0	3.94	12.2	89.6	31.0	34.6	47.0	hgb AS
T-79	85.1	>1	4.5	4.3	4.25	12.1	90.4	28.5	31.5	44.9	ß -thalassaemia minor
T-80	68.5	>1	5.8	-	3.9	8.0	65.4	18.8	31.0	32.4	ß -thalassaemia minor
T-81	62.4	>1	3.8	3.2	4.74	7.3	56.3	15.4	27.3	39.2	ß -thalassaemia major
T-82	65.7	>1	4.2	1.0	5.47	9.8	60.1	17.9	29.8	36.6	ß -thalassaemia minor
T-83	51.0	<1	3.2	43.0	4.71	12.4	82.8	26.3	31.8	42.9	hgb AS
T-84	3.9	18.0	4.1	78.0	3.94	12.2	89.6	31.0	34.6	47.0	Hb S/β Th
T-85	81.7	>1	3.9	2.5	4.46	11.2	81.8	25.1	30.7	48.0	ß -thalassaemia minor
T-86	80.4	>1	8.1	3.0	5.70	9.4	58.2	16.5	28.3	37.2	ß -thalassaemia minor
T-87	85.1	>1	4.5	4.3	4.25	12.1	90.4	28.5	31.5	44.9	ß -thalassaemia minor
T-88	83.6	>1	5.6	-	6.06	11.9	65.1	19.6	30.1	17.4	ß -thalassaemia minor
T-89	79.2	>1	6.3	-	5.0	10.2	57.1	24.7	25.2	16.2	ß -thalassaemia minor
T-90	86.2	>1	4.2	-	4.5	9.01	61.0	28.4	19.02	14.3	ß -thalassaemia minor
T-91	82.3	>1	5.9	-	4.72	7.4	57.2	15.7	27.4	41.7	ß -thalassaemia minor
T-92	22.0	12.0	35	63.0	4.18	11.7	82.3	28.5	34.1	47.1	Hb S/β Th
T-93	82.7	>1	5.9	-	4.61	7.0	57.4	15.3	28.4	40.5	ß -thalassaemia minor
T-94	82.3	>1	5.9	-	4.72	7.4	57.2	15.7	27.4	41.7	ß -thalassaemia minor
T-95	83.5	>1	6.5	-	4.89	7.5	56.0	15.3	27.4	46.7	ß -thalassaemia minor
T-96	0.0	13.0	4.2	91.0	4.71	12.4	82.8	26.3	31.8	42.9	Hb S/β Th
T-97	59.0	<1	3.3	43.0	3.94	12.2	89.6	31.0	34.6	47.0	hgb AS

T-98	83.9	>1	4.0	2.3	4.69	7.7	61.4	16.4	26.7	39.4	ß -thalassaemia minor
T-99	86.2	>1	4.4	-	4.5	9.1	61.0	28.0	19.02	14.3	ß -thalassaemia minor
T-100	65.8	>1	6.5	-	4.17	9.2	66.7	22.1	33.1	26.2	ß -thalassaemia minor
T-101	65.7	>1	4.2	1.0	5.47	9.8	60.1	17.9	29.8	36.6	ß -thalassaemia minor
T-102	80.9	>1	3.1	4.0	3.98	6.8	64.8	17.1	26.4	50.7	ß -thalassaemia minor
T-103	30.0	18	4.5	55.0	4.18	11.7	82.3	28.0	34.0	47.1	Hb S/β Th
T-104	0.0	100	3.2	44.0	3.94	12.2	89.6	31.0	34.6	47.0	SCD
T-105	85.1	>1	4.5	4.3	4.25	12.1	90.4	28.5	31.5	44.9	ß -thalassaemia minor
T-106	68.5	>1	5.8	-	3.9	8.0	65.4	18.8	31.0	32.4	ß -thalassaemia minor
T-107	62.4	>1	3.8	3.2	4.74	7.3	56.3	15.4	27.3	39.2	ß -thalassaemia major
T-108	58.4	>1	6.0	-	4.71	12.4	82.8	26.3	31.8	42.9	ß -thalassaemia minor
T-109	82.3	>1	5.9	-	4.72	7.4	57.2	15.7	27.4	41.7	ß -thalassaemia minor
T-110	84.2	>1	4.2	-	4.9	8.8	65.4	17.9	27.4	45.0	ß -thalassaemia minor
T-111	84.6	>1	5.4	-	5.33	16.3	88.0	30.6	34.8	44.0	ß -thalassaemia minor
T-112	82.3	>1	5.9	-	4.72	7.4	57.2	15.7	27.4	41.7	ß -thalassaemia minor
T-113	65.7	>1	4.2	1.0	5.47	9.8	60.1	17.9	29.8	36.6	ß -thalassaemia minor
T-114	57.0	<1	4.3	91.0	4.71	12.4	82.8	26.3	31.8	42.9	hgb AS
T-115	79.4	>1	12.9	-	5.44	10.2	61.8	18.8	30.4	36.2	HbE
T-116	79.4	>1	5.9	4.0	6.59	11.5	59.2	17.5	29.5	37.5	ß -thalassaemia major
T-117	86.2	>1	3.2	-	4.5	9.1	61.0	28.4	19.02	14.3	ß -thalassaemia major
T-118	79.7	>1	2.2	3.0	3.31	6.6	86.1	28.7	33.3	51.3	ß -thalassaemia major
T-119	0.0	6.2	4.3	91.0	4.18	11.7	82.3	28.5	34.1	47.1	Hb S/β Th
T-120	0.0	13.0	3.1	92.0	4.71	12.4	82.8	26.3	31.8	42.9	SCD

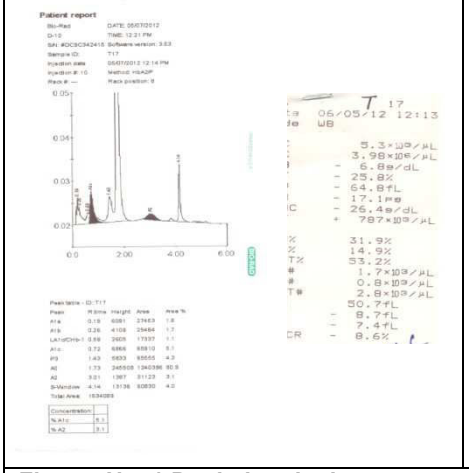
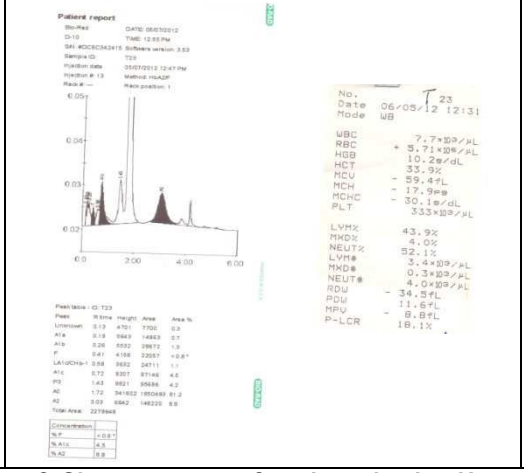
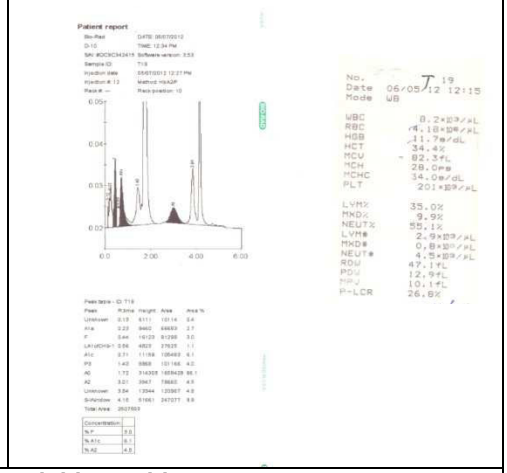
Sickle Cell Anaemia	Beta-Thalassaemia Trait	Sickle / Beta- Thalassaemia
		

Figure No. 1 Depicting the haematogram & Chromatogram of patients having Haemoglobinopathies

The Table-4 shows the frequency distribution of 84 subjects having different clinical manifestations at the time of examination and figure No.2 depicts the two subjects with clinical manifestations.

Table No.4

Showing the distribution of the subjects in the samples according presence or absence of some clinical manifestations.

Variables		Numbers	Percentage	Cumulative percentage
Weakness	Present	84	70	70
	Not Present	36	30	100
Pale	Present	98	82.1	82.1
	Not Present	22	17.1	100
Body ache	Present	36	30	30
	Not Present	84	70	100
Jaundice	Present	30	25	25
	Not Present	90	75	100
Bronze skin	Present	54	45	45
	Not Present	66	55	100
Mongoloid Features	Present	72	60.71	60.71
	Not Present	48	39.29	100
Liver enlargement	Present	14	12.14	12.14
	Not Present	106	87.86	100
Spleen enlargement	Present	18	15	15
	Not Present	102	85	100
Diabetes	Present	7	6.42	6.42
	Not Present	113	93.58	100
Heart problem	Present	6	5	5
	Not Present	114	95	100

Girl of 12 year with sickle cell anaemia

Boy of 10 year with Beta-thalassaemia

Figure No.2

shows the clinical manifestation of haemoglobinopathies:-
Girl with sickle cell anaemia having vasculararocclusion in right leg.
Boy with beta-thalassaemia having discoloured skin and splenomegaly

The Table 5 shows the mean value height, weight and Body Mass Index BMI of subjects having haemoglobinopathies .

Table No. 5 ***shows the distribution of height , weight and BMI according to the type of haemoglobinopathies in the subjects.***

Disease	Height (in cm)	Weight (Kg)	BMI
ß-thalassaemia minor	150±10	40±9	17.78±1.07
ß-thalassaemia major	117.1±16	22±6.8	16.04±2.0
Hb S/β Th	156±9	38±8.6	15.61±2.2
Hgb AS	160±6	35±5.9	13.6±2.4
SCD	120±19	28±7.1	19.4±1.9
HbE	153±2	42±1	17.94±1.8

Table 6 and 7 represents the frequency distribution of blood rate and chelation therapy in 33 patients.

Table No. 6 ***shows the frequency of blood transfution given to 33 patients.***

Frequency of blood transfusion	Number of patients	Percentage	Cumulative Percentage
<2 weeks	10	30.30	30.30
2-4 weeks	12	36.36	66.66
>4 weeks	11	33.34	100

Table No. 7 represents the treatment of chelators.

Frequency of iron chelator Desferol/Asunra	Number of patients	Percentage	Cumulative Percentage
<3 times/ weeks	9	27.27	27.27
3-4 times/ weeks	11	33.33	60.60
>4 times /weeks	13	39.40	100

The table-8 and 9 show the level of copper and zinc in 30 control 120 patients of different haemoglobinopathies.

Table No. 8 ***Showing the concentration of Copper (Cu) and Zinc (Zn) in control subjects.***

Number of control	Copper (Cu) (µg/dl)	Zinc (Zn) (µg/dl)	Ratio (µg/dl)
C1	101.75	96.44	1.05506
C2	100.80	91.52	1.101399
C3	90.45	84.23	1.073845
C4	95.63	88.25	1.083626
C5	87.18	80.97	1.076695
C6	110.64	99.83	1.108284
C7	103.42	96.73	1.069162
C8	97.65	89.99	1.085121
C9	92.71	85.98	1.078274
C10	89.20	82.90	1.075995
C11	101.75	96.44	1.05506
C12	100.80	91.52	1.101399
C13	90.45	84.23	1.073845
C14	95.63	88.25	1.083626
C15	87.18	80.97	1.076695
C16	110.64	99.83	1.108284
C17	103.42	96.73	1.069162
C18	97.65	89.99	1.085121
C19	92.71	85.98	1.078274

C20	89.20	82.90	1.075995
C21	101.75	96.44	1.05506
C22	100.80	91.52	1.101399
C23	90.45	84.23	1.073845
C24	95.63	88.25	1.083626
C25	87.18	80.97	1.076695
C26	110.64	99.83	1.108284
C27	103.42	96.73	1.069162
C28	90.45	84.23	1.073845
C29	95.63	88.25	1.083626
C30	87.18	80.97	1.076695

The mean value of copper is 96.733 µg/dl ; zinc 89.503 µg/dl; ratio of copper / zinc 1.08057 in control.

Table No. 9 ***Represents the values copper & Zinc in subjects with different haemoglobinopathies.***

Number of subjects	Copper (Cu) (µg/dl)	Zinc (Zn) (µg/dl)	Ratio (µg/dl)
T-1	162.10	38.65	4.194049
T-2	155.20	36.97	4.197998
T-3	157.70	37.89	4.162048
T-4	167.43	40.19	4.165962
T-5	170.46	41.09	4.148455
T-6	164.33	39.51	4.1592
T-7	151.19	36.17	4.179983
T-8	161.65	38.91	4.154459
T-9	160.33	39.10	4.100512
T-10	158.49	37.87	4.185107
T-11	165.52	40.11	4.126652
T-12	153.49	36.91	4.158494
T-13	162.50	39.40	4.124365
T-14	158.95	38.33	4.146882
T-15	154.66	37.32	4.144159
T-16	169.35	40.67	4.164003
T-17	173.42	41.90	4.138902
T-18	166.54	39.88	4.176028
T-19	159.89	38.17	4.188892
T-20	167.48	40.06	4.180729
T-21	153.65	37.01	4.151581
T-22	173.40	42.30	4.099291
T-23	159.35	38.70	4.117571
T-24	161.88	39.10	4.140153
T-25	158.44	38.30	4.136815
T-26	160.99	38.77	4.152437
T-27	171.94	41.59	4.134167
T-28	168.63	40.49	4.164732
T-29	157.29	37.96	4.143572
T-30	170.81	41.08	4.157984
T-31	156.59	37.89	4.132753
T-32	164.82	40.05	4.115356
T-33	159.61	38.44	4.152185
T-34	155.60	37.33	4.168229
T-35	163.48	39.67	4.120998
T-36	159.95	38.87	4.114999
T-37	165.30	40.17	4.115011
T-38	164.80	39.45	4.17744
T-39	172.65	41.94	4.116595
T-40	173.68	42.14	4.1215
T-41	164.26	39.49	4.159534
T-42	157.34	38.03	4.13726
T-43	161.28	38.75	4.162065

T-44	157.69	38.15	4.133421
T-45	170.89	41.29	4.138775
T-46	174.54	42.25	4.131124
T-47	168.94	40.63	4.158011
T-48	159.98	38.91	4.111539
T-49	163.75	39.81	4.113288
T-50	165.76	40.35	4.108055
T-51	156.10	37.42	4.171566
T-52	148.66	35.44	4.194695
T-53	149.65	36.17	4.137407
T-54	153.10	37.10	4.126685
T-55	160.38	39.05	4.107042
T-56	164.94	39.84	4.14006
T-57	159.35	38.48	4.141112
T-58	169.19	40.93	4.133643
T-59	157.87	38.47	4.103717
T-60	165.37	40.27	4.106531
T-61	156.59	37.89	4.132753
T-62	164.82	40.05	4.115356
T-63	159.61	38.44	4.152185
T-64	155.60	37.33	4.168229
T-65	163.48	39.67	4.120998
T-66	159.95	38.87	4.114999
T-67	165.30	40.17	4.115011
T-68	164.80	39.45	4.17744
T-69	172.65	41.94	4.116595
T-70	173.68	42.14	4.1215
T-71	164.26	39.49	4.159534
T-72	157.34	38.03	4.13726
T-73	161.28	38.75	4.162065
T-74	157.69	38.15	4.133421
T-75	162.10	38.65	4.194049
T-76	155.20	36.97	4.197998
T-77	157.70	37.89	4.162048
T-78	167.43	40.19	4.165962
T-79	170.46	41.09	4.148455
T-80	164.33	39.51	4.1592
T-81	151.19	36.17	4.179983
T-82	161.65	38.91	4.154459
T-83	160.33	39.10	4.100512
T-84	158.49	37.87	4.185107
T-85	165.52	40.11	4.126652
T-86	153.49	36.91	4.158494
T-87	162.50	39.40	4.124365
T-88	154.66	37.32	4.144159
T-89	169.35	40.67	4.164003
T-90	173.42	41.90	4.138902
T-91	166.54	39.88	4.176028
T-92	159.89	38.17	4.188892
T-93	167.48	40.06	4.180729
T-94	153.65	37.01	4.151581
T-95	173.40	42.30	4.099291
T-96	159.35	38.70	4.117571
T-97	161.88	39.10	4.140153
T-98	158.44	38.30	4.136815
T-99	160.99	38.77	4.152437
T-100	171.94	41.59	4.134167
T-101	168.63	40.49	4.164732
T-102	161.28	38.75	4.162065
T-103	157.69	38.15	4.133421
T-104	170.89	41.29	4.138775
T-105	174.54	42.25	4.131124

T-106	168.94	40.63	4.158011
T-107	159.98	38.91	4.111539
T-108	161.28	38.75	4.162065
T-109	157.69	38.15	4.133421
T-110	162.10	38.65	4.194049
T-111	155.20	36.97	4.197998
T-112	157.70	37.89	4.162048
T-113	167.43	40.19	4.165962
T-114	170.46	41.09	4.148455
T-115	164.33	39.51	4.1592
T-116	151.19	36.17	4.179983
T-117	161.65	38.91	4.154459
T-118	160.33	39.10	4.100512
T-119	158.49	37.87	4.185107
T-120	165.52	40.11	4.126652
The mean value of copper is 162.469 (µg/dl) ; zinc is 39.182 (µg/dl); Ratio of copper / zinc is 4.1468 (µg/dl).			

DISCUSSION

The present study was conducted in 500 volunteers of Sindhi community, out of which 140 NESTROFT and solubility test positive samples were further analyzed with the help of HPLC D-10 for different types of haemoglobinopathies. 120 subjects were having haemoglobinopathies (thalassaemia and sickle cell anaemia or combination of thalassaemia/sickle cell anaemia. Heamoglobinopathies (sickle & thalassaemia) were seen in Sindhi community, this may be due to high rate of consanguineous marriage [42] .The gender discrimination is seen in this study since the number of male subjects 88 (73.33%) constituted higher than females 32 (26.67%), This could be explained by the fact that people in developing countries are more concerned about male than female children. Since men are basically considered as bread earner in developing countries. The mean value of Hb was 9.88 gm/dl, Total RBC Count 4.75 mill/cumn, MCV 68.89 fL, MCH 21.80 pg, MCHC 29.42 g/ dl and RDW 38.63 % that shows that RBC, Hb, MCV, MCH,MCHC were lower as compared to control where as RDW was higher . It is in agreement with other studies conducted on these parameters [43-44]. The BMI in the present study was low as compared to standard. In the case of beta-thalassaemia minor the height , weight and BMI were 150±10,40±9,17.78±1.07;ß-thalassaemia major 117.1±16,22±6.8,16.04±2.0; HbS/βTh 156±9, 38±8.6, 15.61±2.2 ; Hb AS 160±6, 35±5.9,13.6±2.4; SCD 120±19,28±7.1,19.4±1.9; HbE153±2,42±1,17.94±1.8. The present sample shows that subjects with haemoglobinopathies were underweight and having growth retardation these findings co-inside with the other studies[19, 45-46].

The clinical findings like paleness, weakness, body ache,spleen enlargement and liver enlargement in the samples indicate that subjects were under poor treatments due to low socio-economic condition and they were highly prone to infections [2]. These clinical complications are more related to zinc and copper as documented in many studies [47-50]. Six patients were suffering from heart diseases in the sample group, It is documented that excess of iron is deposited in the human body due to blood transfusion and not removed thus lead to death due to iron over loading of myocardium [51-52]. In a study it was found that patients with thalassaemia major showed marked reduction in contractile state and milder left Ventricle Function (LVF) than in thalassaemia intermedia. It is important to notice that classical changes of untreated thalassaemia major are now regularly seen only in countries without resources to support long term transfusion programs [53-54]. The study shows higher level of copper / zinc ratio 4.1468 (µg/dl) in 120 patients of haemoglobinopathies where as in 30 control it is copper / zinc

1.08057(µg/dl). It shows lower level of zinc as compare to copper in haemoglobinopathies The similar observation were observed by [42,48,55-61]. The growth retardation, puberty disorder, dysgeusia and clinical manifestation were observed in the samples (thalassaemics & sickle cell anaemia) mainly due to low level of zinc the similar observation observed by [62-76]. The chelation therapy has many complications like growth impairment, endocrinophathy, hypogonadism etc [77-78]. Desferrioxamine(DFO) the chelator affects the plasma level of zinc and copper and cobalt [30,79-81]. These elements are more likely to be chelated in presence of reduced iron level [82]. Increased glomerular filtration rate of Zn is responsible for hyperzincuria resulted from hemolysis of red blood cells [23, 42. 50, 64, 71, 83-89].

It has been reported in experimental study that plasma somatomedin-c correlates with zinc status in animal and activity of soamtomedin-C decreases as a result of dietary zinc deficiency[56,90-91] .Zinc supplementation has shown positive effect on the hepatic synthesis of somatomedin-C [92-94]. It has been shown in many studies that zinc deficiencies are allied with thalassaemia [40,4164,80,83,90,95-97],may be due to reduced zinc intake and chelation therapy[42,80,87,90,98-104]. Zinc deficiency plays important role in the growth retardation and sexual development in thalassaemic [31]. The close relation between serum cu and iron can be explained by the importance of copper containing enzymes and co-factors for iron absorption and the effect of copper on the release of iron from the body stores as well the utilization of iron in haemoglobin synthesis [105-106]. Hypercupraemia is seen in haemochromatosis the principle complication in thalassemia [44-107]. According to some studies zinc and copper level are shown higher in haemoglobinopathies (thalassaemics & Sickle cell anaemia [108-109] , which may be due to blood transfusion from healthy donor [110] or due to cirrhotic changes owing to hemosiderosis or because of abnormal rate of glomerular filtration of zinc seen in chronic hemolysis [111] and may be due to impaired zinc and copper utilization in tissue in the pathogenesis [91]. Wide range of discrepancies were observed in various reports these different findings may be due to variation in subject age, different transfusion and chelators therapies [112], or due to anorexia, nutritional status, psychological problems and different metabolic and endocrine complications [113-114]. Zinc concentration is higher in RBC [64]. During transfusion of blood a considerable proportion of transfused cells are destroyed [43], patients who are transfusion dependent will have higher serum zinc as compared to control subjects. Liver is storage organ for zinc[115], due to iron overload; oxygen free radical may induce peroxidative damage[116], thus increased serum zinc from damaged hepatocytes[115]. It was hypothesis that variation of serum zinc level may be due to leukocyte endogenous mediator (LEM) which has the property of mobilizing zinc from its stores in liver and other tissue to the serum[117].

CONCLUSION

This study reveals that zinc deficiency is common in haemoglobinopathic patients (thalassemia and sickle cell anaemia) but there is no copper deficiency. It was observed that about 24% of Shindhi community is suffering from haemoglobinopathies. It is observed low zinc level in haemoglobinopathy is one of the factor causing growth retardation and other complication like diabetes, liver disorder and heart diseases. With dietary intake of zinc and transfusion of healthy blood will reduce these complications. It is suggested that measurement of serum zinc/ copper levels be a routine part of management of all patients of haemoglobinopathies particularly those who are treated by chelation therapy.

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