Original Article

PREVALENCE OF BETA-THALASSEMIA TRAIT IN QUETTA CITY, CROSS SECTION STUDY

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ABSTRACT: Identifying the prevalence of Beta Thalassaemia and its carriers in the population of Quetta city.

METHOD: Rapid methods of screening like Osmotic Fragility Test (OFT),Mean Cell Volume(MCV), Mean Cell Hemoglobin(MCH), Red Blood Cell (RBC) count and Hemoglobin (Hb) estimation was performed on hematology analyzer, For discrimination Shine and Lal formula was used and morphology of erythrocytes were studied. For these fresh venous blood samples were obtained from different blood banks and hospitals of Quetta city. Using cross section method, a total of 570 blood samples were obtained, Among those 59% were male and 41 % were female.

RESULT: Prevalence of Beta thalassemia in population of the city was 6.5% in 2012. The median age was 20 years. 72% were male and 27% were females victims. Ethnic group distribution Pathans, Baloch, Hazara, Afghan refugees and miscellaneous were 41%, 30%, 5%, 11% and 14% respectively. Hematological parameters, OFT observation, blood become turbid in all cases, the mean Hb level were not too much high i.e 10.2 g/dl , MCH (mean) was 18.2(pg) , RBC count 5.9 (10^6/L) mean, MCV was 56(fl) ,In microscopic examination, erythrocytes contained high numbers of microcytosis. RBC morphology also show basophilic stippling and hypochromic.

CONCLUSION: OFT not statistically tested for discriminating between anemia, Iron deficiency and Beta thalassemia. Due to absence of a single pathognomonic findings that covers all variants there is need of a set of analysis parameter which give reliable and quick results. On the basis of the evidence provided there is a need for immediately implementing proper screening at grass root level as per guidelines issued by the WHO. Further there is the need of law implemented for couples before they are given approval to get married.

KEY WORDS: Single tube test, pathognomonic, Screening , Quetta, Gene.

INTRODUCTION:

Thalassemia is a result of globin chain disorders that are concerned with the lack of production of alpha or beta globin chains. As a result defective haemoglobin is synthesized[1]. Failure to synthesize either the alpha or the beta chain impairs the production of the normal hemoglobin, thalassemia syndrome is a genetic defect which can be seen in any ethnic group and pass to next generation[2]. The screening of these congenital syndrome is not an easy job, mainly because of heterogeneity of Beta Thalassaemia and the absence of a single pathognomonic finding that covers all variants.

Different types of screening methods and tests are to find out this single gene disorder in general population and they are based on serological testing.

Single test tube or osmotic fragility test (OFT) is the method used for the screening of thalassaemia by many[5]. OFT was first defined by Silvestroni and Bianco in 1940[3]. The validity of this Single tube test is also Proved by Jason Chow *et al*[4]. with The advancement

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in technology and availability of electronic cell counters gadget for the measurement of mean cell volume(MCV) and mean cell hemoglobin(MCH) the use of OFT has been decreased over the years. But it is still useful to minimize the economical load, but in order to get accurate results there is a need of further analysis.

OFT is based on the measure of red blood cell lysis as a function of osmotic

stress. When RBCs are kept in hypotonic solutions, they start to take in water

osmotically[5]. As a result the cell begins to start swelling when an optimized volume is reached the cell membrane ruptures and hemoglobin are released.

It is essential to determine how common single gene syndrome is in any place with the help of below mentioned parameters. It is very difficult to engage the government or health service providers, they ask for the potential number to be decided whether it is worthwhile for them to get involved in the area. But in Quetta district according to our information, no definite national screening program is set to determine Beta Thalassaemia and its carriers till date. The key objective of current study is to determine Beta Thalassaemia and its carriers in the city, and to use the most economic, fast and accurate method for mass screening of Beta Thalassaemia.

LOCATION:

Quetta district is considered an urban district and is located in the northwest of Balochistan at 30°10′N 67°00′E coordinates. Quetta is the capital of Balochistan province in Pakistan . The total area of Quetta is 1,024 square mile approximately, located at the west edge of Pakistan at an altitude of 5,260 ft above sea level and the 6th largest city of Pakistan[6]. City is surrounded by mountain ranges namely Chiltan, Takatoo, Murdar and Zarghun[7]. The city population is about 759,894 in 2012[8]. Different types of tribes are living in the city with smaller minority groups[9], while the city is overwhelmingly with muslim.

SAMPLE COLLECTION:

The month of April 2012 was selected for carrying out the experiment and analysis was completed in five months. In hospitals many people came for blood analysis, and in blood banks for blood donation. For the purpose of collecting blood samples we selected one hospital and two blood banks in the city.

METHOD AND MATERIAL:

1. 10 - 15 ml of whole blood collected in EDTA is titrated in a glass test tube (100 x 10 mm) containing 4 ml of 0.36% buffered saline solution.
2. Shake the tube and left at room temperature for 30 minutes.
3. Shake the tube again and immediately hold the tube in front of a piece of paper with text.

METHOD OF MAKING OFT:

0.36 % buffered saline solution.

Dilute 10% stock solution of sodium chloride *(90 g)*, Disodium hydrogen phosphate *(13.65 g)* and sodium Di hydrogen phosphate(2.43 g) in 1000 ml of distilled water*(pH 7.4)*. The original method *(provided by Prof. Ida Bianco)* uses the Tyrode's solution, diluted 4:10 with distilled water[10].

Tyrode's solution *(*1Litre) NaCl=8.2 grams (g), KCl=0.2 g, $MgCl_2.2.6\ H_2O$ = 0.2 g, $CaCl_2.2.2\ H_2O$ = 0.2 g, $NaH_2PO_4.H_2O$ = 0.1 g and $NaHCO_3$ = 0.05 g. Tyrode's solution should be stored at 4 °C and the work solution must be prepared a fresh before its use[4]. mean cell volume and hemoglobin estimation was performed on automatic cell counter gadget. RBCs morphology is common finding under compound microscope using colour dyeing for thalassemia[21].

STATISTICAL ANALYSIS:

Data obtained from above mentioned parameters was subjected to statistical analysis through (S.P.S.S version 14 p.c software).

SELECTION CRITERIA:

In order to avoid human error and to represent data on the basis of merit, random generation number method was used. Quetta city consists

of two towns. Chiltan town has 22 union councils and Zarghoon town has 23 union councils. For the population of 1000 one sample was obtained for examination. Volunteers were asked about their health status, and all the participants looked normal at the time of study. From the above mentioned union councils of the city, a total of 516 blood samples were obtained. 27 samples were obtained from persons who are in Quetta for five year but are not permanent resident i.e those who lived in Quetta, for the past five years, but were not registered citizens of Quetta. 27 samples were obtained from Afghan refugees. A Total of 570 samples were obtained in all for current examination.

RESULT:

Prevalence of Beta thalassemia in general population of Quetta city is calculated 6.5% in 2012, which include mild, major, carrier and moderators.

Table 1. Hematological parameters.

Parameters (Mean)	OFT	Hb	MCH	RBC Count	MCV	RBC Morphology
Unit	All solution become turbid	g/dl	27 - 31 picograms/cell	10^6/L	80 - 100 femtoliter	Hypochromia and Microcytosis, Basophilic stippling
Result n=37 (mean)		10.24	18.2	5.9	56.12	

Where OFT = Osmotic fragility test, Hb = Hemoglobin, MCH = Mean Corpuscular hemoglobin, RBC= red blood cell, MCV = Mean corpuscular Volume.

CALCULATION:

Total no of samples = 570/- (340 male, 230 female)
Effected sample = 37/- Percentage =(37/570) X 100 = 6.4912280 (Round fig) = 6.5 %.

Formula Base measurement:

Shine and Lal formula [$(MCV^2 \times MCH)/100$] was used to differentiate iron deficiency from Beta thalassemia[22] validity of this formula also confirm by Zahid hussain *et al* with 92% accuracy[23]. In this current study further confirmation was done by using same formula. According to above mentioned formula if the answer is " <1530" this is beta thalassemia and if the result ">1530" it mean iron deficiency.

Table 2. Gender wise ratio.

Gender	**Total no of sample 570/-**	**Percentage**	**Effected sample =37/-**	**Percentage**
Male	340	59.6 %	27	72 %
Female	230	40.3 %	10	27 %

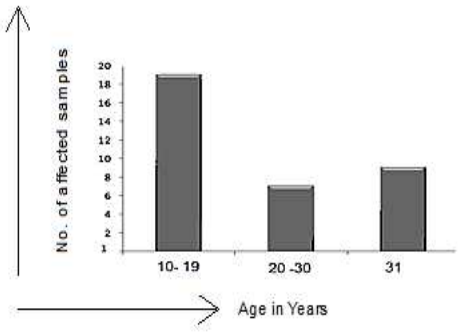
Table 3. Age wise description.

No of sample	Minimum age	Maximum age	Median age	Mode age	Mean Age	Std Deviation
37	9	50	20	12	24.5833	13.28022

Table 4. Age group percentage of beta thalassemia in Quetta district.

Age(Year)	Effected No. of samples	Percentage %
10 – 19	19	54 %
20 – 30	7	20 %
31 – 50	9	24 %

Note: From the age of 31 to 50 volunteers were Moderated,Age of 20 -30 mild beta thalassmia victims, While the age of 10 -19 years patients were victims of major beta thalassemia as shown in table 4.



On X-axis age group distribution, Y -axis show the numbers of affected samples.

Fig 1: Age group distrbution.

Beta thalassemia was found to be more common in Pathans were with 41%, Baloch were 30%, Afghan refugees were 11% and 5% were Hazara, While the rest belonged to other minor ethnic groups. (Table 5)

Table 5. Ethnic distribution pattern of Beta Thalassaemia in a Quetta, district.

Ethnic group	No. of obs	Percentage %
Pathans	15	41
Baloch	11	30
Hazara	2	5
Afghan refugees	4	11
Miscellaneous	5	14

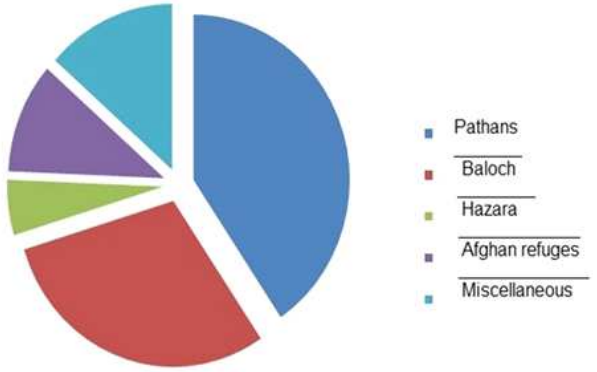


Fig-2: Shows different ethnic group distribution of beta Thalassaemia in a Quetta, city.

DISCUSSION:

OFT:

OFT test was used to minimize the analysis cost. But in areas where iron deficiency anemia and beta-Thalassaemia trait are common, MCV, RBC count ratio can be used to differentiate between iron deficiency, anemia and Beta thalassemia trait[11,12].

Mean Cell Volume:

Low MCV results shows a chance of Beta thalassemia or its carrier[26], But does not ignore the chance of iron-deficiency and microcytic anemia.

Red Blood Cell Count:

RBC count is a test that is used to know the production or life span of the red blood cells. If there are low RBCs it means bone marrow is not able to produce new ones in required order or hemolysis, resulting in an anemia[13].

Mean corpuscular hemoglobin / Hb estimation:

In the Blood of thalassemia affected gender hemoglobin level is decreased as compared to normal[14]. It reflects that the body is unable to distribute the required oxygen throughout the body.

Formula base measurement:

Now a days many formulae have been reported to discriminate between anemia, beta thalassemia trait and iron deficiency[12,24]. Measuring coefficient of variation by mathematical computation after obtaining a result from hematology analyzer is an useful technique for rapidly distinguishing beta thalassaemia.

RBC Morphology:

The use of red blood cell morphology to diagnose beta-thalassemia trait are discussed by many[15]. Generally speaking, the microscopic examination of blood obtained from thalassemic individual has marked numbers of hypochromia and microcytosis. High number of microcytosis confirms the presence of thalassemia trait[16]. Basophilic stippling is normally observed in thalassemic blood samples but it is not seen in iron deficiency blood films, cells are more hemolytic in thalassemia but not during iron deficiency.

CONCLUSION AND RECOMMENDATION:

The above sets of methods are easy to perform, fast, cheap and does not require sophisticated and sensitive equipments. However, it needs careful standardization. It is particularly useful with the electronic cell counters to obtain results, reduce time. The core aim of current study was to determine the rate of beta thalassaemia in city. A cross-sectional study measures prevalent rather than incident cases, the data will always reflect determinants of survival as well as etiology[17]. In advanced countries Governments show keen concern on the screening of population because it is indirectly related to the health issues of humans, like cerebral infarction[27] depression in mother who have thalassemic children[25] and also motivate the nation for screening and inform them why it is beneficial for the country at large. In an under developed country like Pakistan government gives less attention to health sectors and hardly determines the health status, due to the following facts, poor economic condition, natural disasters, poor health policy, lack of proper planning in the said sector. So on the basis of the evidence provided above it easily draws the following conclusions there is a need for immediately implementing proper screening at grass root level as per guidelines issued by the WHO. Identification of any genetic disorder is to reduce the burden on individuals by identifying those at increased risk, thereby enabling individuals to receive information about their personal health[18]. The above systematic data provides a framework and alarming signal that we should also make laws and take a leaf out of neighboring countries like Saudi Arabia and Iran[19,20] there is a law that premarital test for haemoglobinopathies diagnosis is essential for all before they are given approval to get married.

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