

Investigation of Genetic Epidemiology of Metabolic Compromises in β Thalassemia Minor Mutation: Phenotypic Pleiotropy

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Abstract—Human genome is not only the evolutionary summation of all advantageous events, but also houses lesions of deleterious foot prints. A single gene mutation sometimes may express multiple consequences in numerous tissues and a linear relationship of the genotype and the phenotype may often be obscure. β Thalassemia minor, a transfusion independent mild anaemia, coupled with environment among other factors may articulate into phenotypic pleiotropy with Hypcholesterolemia, Vitamin D deficiency, Tissue hypoxia, Hyper-parathyroidism and Psychological alterations. Occurrence of Pancreatic insufficiency, resultant steatorrhoea, Vitamin-D (25-OH) deficiency (13.86 ng/ml) with Hypcholesterolemia (85mg/dl) in a 30 years old male β Thal-minor patient (Hemoglobin 11mg/dl with Fetal Hemoglobin 2.10%, Hb A2 4.60% and Hb Adult 84.80% and altered Hemogram) with increased Para thyroid hormone (62 pg/ml) & moderate Serum Ca^{+2} (9.5mg/ml) indicate towards a cascade of phenotypic pleiotropy where the β Thalassemia mutation be it in the 5' cap site of the mRNA, differential splicing etc in heterozygous state is effecting several metabolic pathways. Compensatory extramedullary hematopoiesis may not coped up well with the stressful life style of the young individual and increased erythropoietic stress with high demand for cholesterol for RBC membrane synthesis may have resulted in Hypcholesterolemia. Oxidative stress and tissue hypoxia may have caused the pancreatic insufficiency, leading to Vitamin D deficiency. This may in turn have caused the secondary hyperparathyroidism to sustain serum Calcium level. Irritability and stress intolerance of the patient was a cumulative effect of the vicious cycle of metabolic compromises. From these findings we propose that the metabolic deficiencies in the β Thalassemia mutations may be considered as the phenotypic display of the pleiotropy to explain the genetic epidemiology.

According to the recommendations from the NIH Workshop on Gene-Environment Interplay in Common Complex Diseases: Forging an Integrative Model, study design of observations should be informed by gene-environment hypotheses and results of a study (genetic diseases) should be published to inform future hypotheses. Variety of approaches is needed to capture data on all possible aspects, each of which is likely to contribute to the etiology of disease. Speakers also agreed that there is a need for development of new statistical methods and measurement tools to appraise information that may be missed out by conventional method where large sample size is needed to segregate considerable effect.

A meta analytic cohort study in future may bring about significant insight on to the title comment.

Keywords—Genetic disease, Genetic epidemiology, Heterozygous, Phenotype, Pleiotropy, β Thalassemia minor, Metabolic compromises.

I. INTRODUCTION

THE central dogma of molecular biology infuses the transcriptional and translational events in between the genotype (DNA) and the phenotype (expressed protein). These events of gene expression are carefully regulated at the gene level (DNA) and also at the transcript level (RNA). The final outcome of the information pathway, ie the protein then undergo episodes of post translational modification as glycosylation, editing etc in specialized cellular compartments to achieve its full functional potency. The secondary transcript (RNA) also halts for several post transcriptional modifications as splicing, addition of 3' Poly-A tail and 5' Cap among others. All these above phenomenon taking place during information processing makes the overall pathway a little bit flexible to incorporate necessary amendment (? mutations) which may have an evolutionary significance.

β Thalassemia mutations have been identified that affect most of the above mentioned stops or halts in the information streaming. Some of the notable mutations [1],[2] can be mentioned as follows: transcription mutation of the promoter sequence, upstream to the β globin gene, for example -88 (C→T) β^+ , 5'cap site mutation affecting the binding of mRNA to the small subunit of ribosome or transcription rate for example the β^+ mutation of capsite (A→C) expressing as mild anaemia in Asia and India, RNA splice site mutation in the splice donor and splice acceptor site leading to activation of cryptic splice sites and abnormal amino acid sequence in synthesized protein for example IVS 1-1 (G-T) and IVS 1-5 (G-C) are common in β^0 and β^+ mutations respectively. Deletion of 25bp at IVS-1 3' that affects RNA splicing in Indians. Addition of a poly A tail in the 3' site of mRNA signals transcription termination and mutations in the consensus 5'AAUAA 3' results into longer run on transcripts and consequent β^+ mutation of β globin. At the translation level β globin mutations, as the mutation in initiation codon, insertions and deletions, leading to premature termination codons etc has been described.

In heterozygous state these mutations result into β Thalassemia minor with no life threatening complications but lowering the quality of life to significant extent. The present study reports phenotypic effect of β Thalassemia minor mutation on several metabolic pathway and for the first time evaluates β Thalassemia minor mutation in the light of

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pleiotropy a phenomenon which recognize that most genes may have multiple and qualitatively distinct functions.

II. CLINICAL OBSERVATION AND METHOD

A. Presentation

A thirty one year old male was seeking medical help in a tertiary health care centre of urban India for soaring exhaustion, irritability, episodic insomnia, depression, weight gain. The patient had a major complaint for bone pain throughout the body, the media sternum and flanks. Hb, TC, DC, ESR, PP and F Sugar were advised by the physician. Hb was 13.8 mg/dl, TC of WBC was 6900/cmm, DC were normal and 1st hour ERS was 08mm (Westergren) and occurrence of infection thus ruled out. F Sugar was 85mg/dl and PP was 95mg/dl. Only short term NSAIDs with physical exercise was advised. After three months another visit with some abdominal distress along with an enhancement of previously mentioned complaints of bone pain, irritability etc prompted LFT, RA, lipid profile, HBSAg and Chest PA view. None of the organs were palpable and no complaints of acidity etc were made. USG-whole abdomen revealed moderate hepatomegaly (15.7 cm), C.B.D and P.V within normal dimensions with otherwise normal impression. RA, HBSAg were negative, LFT was normal with all enzymes WNR and normal A:G and Total bilirubin 1.0 mg/dl. Chest X ray was insignificant. Total Cholesterol being 140mg/dl with TG WNR with good HDL, the lipid profile declared to be normal in spite of moderately low total cholesterol. Only Vitamin E as Tocopheryle acetate 400 mg OD after the last meal (night) for 45 days was advised with some physical exercise and distressing. During a follow-up after 2 months bone pain was not subsided. The patient complained of lower quality of life including decrement in professional performance in workplace, depression, profuse sweating and change in bowel habits with episodes of nocturia. The patient had no pallor or icterus or incidents of fever. CRP, Glycosylated Hemoglobin, kidney function panel including Ca+2, Thyroid profile and ECG were done. Serum calcium was moderately low at 8.6 mg/dl whereas other parameters as Urea, Creatinine, Electrolytes WNR and ECG was insignificant with normal rhythms. A review of USG showed mild splenomegaly (125 mm), HbA1c was 5.60%. The thyroid panel including TSH, FT3, FT4 were WNR. Regular examination of stool showed excess mucus, starch and fat globules. During this period the patient complaint of abdominal distress and discomfort after meal. Stool parasite and occult blood for three consecutive days were done. Only one episode of presence of Giardia in stool was noticed and other results were insignificant. The patient was developing strong signs of depression, irritability, lack of concentration, high anxiety, mood swings, loss of appetite and emaciation. Commercial preparation equivalent to 500mg elemental calcium with 250 IU of vitamin D3, OD for one month, Metronidazole 400 mg three times after meal for five days and Domperidone with Pantoprazole in the morning for 30 days were advised. Anti inflammatory medication with tranquilizers were prescribed if condition doesn't get better. After one month of medication there was no improvement of symptoms

. Rather abdominal tension, cholic, abnormal bowel, polyuria, loss of appetite, psychological stress and weight loss continued. Mental anxiety, bone pain and other unsubsidized symptoms prompted the patient to follow up for medical advice. A thorough appraisal was started. The patient was nevertheless desperately prepared to undergo a thorough evaluation to get rid of the symptoms that was lowering his quality of life. Blood tests including Complete hemogram with haematological indices, Liver function test panel, Kidney function test panel, Lipid profile were done. CRP, RA, Anti CCP antibody were evaluated for suspected rheumatic bone pain. Hb1Ac, PP and F Glucose, TSH, PTH with Ca+2 etc were advised to evaluate endocrine condition. HbSAg, HCV, Retrovirus for HIV were advised for blood borne viral assay. Vitamin B 12, Folic acid and Vitamin D (25 OH) in serum were done for nutritional profiling. Regular examination of Urine and Stool were also done. Imaging studies included USG, KUB and MRCP. Hemoglobin was 12gm %, MCV and MCH was abnormal with marked erythrocytosis and hypochromic microcytosis. Reticulocyte count an important parameter was found to be 1.08 %. Serum potassium was 5 mg/dl with otherwise normal KFT. Total bilirubin was 3 mg/dl with marked increase in SGPT (Twice of the normal range), total protein was 6 mg/dl with normal A:G. Total cholesterol reduced to 85 mg/dl. Alkaline phosphatase was moderately increased but Gamma glutamyl transpeptidase was normal. CRP, RA was absent, Anti CCP antibody was < 7 U/ml excluding rheumatic origin of bone pain. ESR was found to be 02 mm/hr (Westergren, normal range 3 – 5 mm/ first hour). TSH was normal but Para thyroid hormone was in upper limit (62 pg/ml), Ca+2 was 9.5 mg/ml. Hb1Ac was just above 5 % with normal PP and F Glucose. HbSAg, HCV, Retrovirus for HIV were negative in blood borne viral assay. Serum Vitamin B 12 level was found to be 466 pgm/ml (Normal level being 200 – 900 pg/mL), Folic acid level was 14.15 ngm/ml (Normal level being 2.7 - 17.0 ng/mL) but Vitamin D (25 OH) was 13.86 ngm/ml (Normal level being 30.0 to 74.0 ng/mL) revealing severe deficiency without pernicious anaemia. Stool RE showed starch and fat globules with excess mucus. Urine RE was otherwise normal besides Ca+2 crystals (++) . USG abdomen was normal besides mild splenomegaly (130 mm). MRCP revealed mild altered signal intensity of pancreatic parenchyma and no hemochromatosis. KUB was insignificant. Low cholesterol, Vit D deficiency, presence of fat globules, high serum Ca+2 prompted through evaluation of fat absorption by advising 72 hours stool fat estimation after dietary fat challenge. 72 hrs stool fat was estimated to be 25.6 gms, much above the normal limit of < 18 gms. Mal absorption of fat resulting into steatorrhoea was confirmed. For evaluation of pancreatic health Endoscopic Ultrasonogram revealed inhomogeneous parenchyma with multiple hyper echoic foci but peripancratic fat planes were well maintained and there was no evidence of focal lesion, dilated pancreatic duct or lymphadenopathy. Major inflammation of pancreas was thus ruled out according to Pancreatitis criteria. Occult blood test of stool was negative. Vitamin D deficiency, complaints of polyuria prompted evaluation of kidney function and 24 hrs urine volume, creatinine clearance, urine osmolarity, 24 hrs

urine proteins were studied. Urine volume was elevated (3560 ml/24hrs) with a normal diet, 132mg/dl protein excreted in 24 hrs, creatinine clearance was 117 ml/min and urine osmolarity was also within normal limits (295 m mol/kg). High urine volume prompted evaluation of serum aldosterone which was found to be within normal range 304.99 pg/ml. Serum erythropoietin and homocysteine were also evaluated and found to be 22.80 mIU/ml and 11.97 umol/L respectively which are within normal limits. Persistent bone pain with absence of rheumatic markers prompted evaluation of bone mineralization by DEXA scan of the neck of femur and the spine. T score of minus 1 observed for both the sites indicating osteopenia of neck of femur and spine as well. Altered PCV, MCV and MCH, decrement in Hb% with marked erythrocytosis and persistent symptoms prompted Hemoglobin HPLC which revealed presence of Fetal Hemoglobin (2.10 %), HbA2 (4.60) and Hb Adult (84.80 %) thus confirming Thalassemia minor and excluding Thalassemia intermedia. TIBC was normal ANA was negative.

B. Method of Clinical Investigation

Hematological investigations were done following standard laboratory methods. Serological studies as CRP, RA, HBsAg were performed by serodiagnostic agglutination kits. Anti CCP antibody, TSH, T3, T4, ANA, EPO, Aldosterone etc were done either by quantitative ELISA or Chemiluminescence. For radiological findings of X-ray, Ultrasonography, Bone densitometry, Endoscopic ultrasonography and ECG, MRCP and 72 hours stool fat estimation professional help were sought. Estimation of Bilirubin, SGOT, SGPT, Lipid profile, Urea, Creatinine etc were done by spectrophotometric analysis. High performance liquid chromatography was used for HBA, HbF, HbA1c and HbA2 analysis.

The review of literature was also done vigilantly, mentioning studies and feedback only on β Thalassemia minor cases and clear demarcation of Beta Thalassemia minor and intermedia was maintained.

III. EXPLAINING THE PHENOTYPES

A. Hypcholesterolemia

Cholesterol synthesis by the hepatic parenchyma is a complex metabolic corridor that involves several enzymatic pathways. Hepatic stress may reduce cholesterol synthesis. Transient alteration in A:G ratio may be seen in Thalassemia minors resulting into mild edema. Malabsorption of fatty matter (Fatty acids are source of Acetyl CoA, the building blocks of Cholesterol) due to bile synthesis deficiency may also be a cause of nutritional Hypcholesterolemia. Constant removal of cholesterol for increased haematopoiesis may also escort to the condition [3]. Lipid profile of the Thalassemia minor patients during a thorough evaluation in repeated incidents have disclosed to us Hypcholesterolemia with near normal HDL and TG.

Thalassemia minors thus have a reduced risk of atherosclerosis and its related cardiac complications [4],[5],[6],[7]. Retrospective studies [8] show that the prevalence of myocardial infarction among thalassemia

carriers was much less than expected and in some samples with beta thalassemia minor, myocardial infarction occurs ten years later than in the non-thalassemic subjects. Hypcholesterolemia may also manifest into low blood pressure which may be complicated if chronically present leading to organic ischemia. Thalassemia minors may have an increased risk of gallstones according to some reports [9],[10],[11]. Ursodeoxycholic acid or Tocopherol supplementation are generally advised symptomatically. [12] has examined the plasma lipid pattern of 628 beta-thalassemia trait carriers and 4552 controls and found Total cholesterol and low density lipoprotein (LDL)-cholesterol levels were significantly lower in beta thalassemia trait carriers when compared to controls and suggest that accelerated erythropoiesis and increased uptake of LDL by macrophages and histiocytes of the reticuloendothelial system are the main determinants of low plasma cholesterol levels in heterozygous Thalassemia.

B. Vitamin D deficiency

We have noticed in the Thalassemia minor patient severe Vitamin D deficiency in spite of regular and healthy diet.

Fat soluble vitamin deficiency may be noticed in Thalassemia minors. Vitamin D, its metabolites [13],[14] and Vitamin E may be deficient. Hypcholesterolemia or malabsorption may result into the deficiency. Therefore Calcium metabolism may be inhibited. Evaluation of renal function is thus again indicated since kidneys actively participate in Vit D metabolism. Increased 72 hour stool fat may be also seen, and absence of established Giardiasis etc may therefore indicate malabsorption or celiac according to [15]. Evaluation of Anti TTG antibody and urine D-Xylose may be requested to evaluate malabsorption. Although classical bone abnormalities and visible deformities as such are not seen in Thalassemia minor though several reports of osteological complications [16],[17],[18] in Thalassemia minors have been described. Regular episodes of bone pains, occurrence of mild elevation in Alkaline phosphatase in absence of Aminotransferase elevation may be confounding. Calcium metabolism abnormality and Hyper Calcuria from demineralization may be observed in Thalassemia minors as well [19]. Fluctuation of serum Calcium level from previously moderate decrease and subsequent increment may indicate a bone mineral densitometry by DEXA scan which remains moderately altered (T scores). Osteoporosis in middle age man is by and large unlikely and therefore therapeutic advice is generally not given. Only one study describes osteonecrosis of the femoral head in Thalassemia minor [20]. Similarly after a detail literature review only one report of seronegative oligoarthritis [21] was found. Parathyroid level may be evaluated for secondary hyperparathyroidism due to deficiency of VIT D. If several findings of Ca crystals (++) are present in urine RE, Bisphosphonate therapy may provide relief. Episodes of bone and extensive body pain in Thalassemia minors may compel a physician for evaluation of RA factor as well as Anti CCP antibody in serum which are regularly found to be normal if Rheumatism is absent. Only a single study has related fibromyalgia with Beta Thalassemia minor [22]. More studies are needed to scrutinize osteological

risk factors in Thal minors. Use of NSAIDs, muscle relaxant should be very cautiously advised and over-the-counter consumption by the patient must not be practiced. According to the study of [18] bone mineral density was diminished in the spine (z-score = -0.78, $P < 0.03$) and hip (z score = -0.54, $P = 0.05$) of 11 patients with transfusion independent thalassemia trait and postulated that Thalassemia minor may be a risk factor for osteoporosis.

C. Malabsorption

Hypocholesterolemia, Vitamin D deficiency may have been resulted due to tissue hypoxia of pancreas in Beta Thalassemia minor patient. Short survival of red blood cells in these patients may lead to cellular stress [23]. Thal minor patients exposed to environmental stress may exhibit a significant decrease in the NADPH/NADP ratio. [24] reported presence of severe steatorrhea in thalassemia minor. Reduced oxygen carrying capacity may bring about hypoxic conditions in long-term which is complemented by low blood pressure and environmental stress. Oxidative stress and ischemic condition [25], [26] in the affected may also be higher comparatively [27]. Moderately Reduced GFR within normal limits and in some cases pancreatic insufficiency may be manifested. Secondary hypercalcemia may further complicate the case in some Thalassemia minors via Alpha 1 antitrypsin pathway. Malabsorption, vitamin deficiency, high stool fat; findings of emaciation may thus can be correlated. For confirmation of pancreatic involvement imaging by MRCP and Endoscopic Ultrasonography of pancreas (Gold standard for pancreatic involvement) may be undertaken. Pancreatic enzyme assays are insignificant since only severe inflammation elevate serum amylase, lipase levels.

D. Psychological Alterations

In our case the Beta Thalassemia minor patient had severe irritability, stress intolerance etc which was affecting his social and professional life. Occasional polyuria and nocturia was reported by the patient. In Thalassemia minors psychological alterations may develop in a multifaceted way [28], [29]. Intolerance to stress may affect professional and social life. There may be a sense of fear, anxiety; mental stress leading to depression. Patients may be on the otherhand become violent and suicidal. Thalassemia carrier trait was observed more in suicidal subjects [30]. A reduced central serotonergic neurotransmission through decreased serotonergic (5HT) receptor function is a biochemical mechanism responsible for association between low cholesterol level and psychopathological processes involved in suicidal, aggressive and violent behaviors [31], [32], [33], [34], [35]. In some cases bowel habits may be changed leading to IBS. Constant irritable state of mind may take its toll leading to alteration in day to night ratio of urine output. Polyuria and nocturia may be manifested. Loss of appetite if persistent may lead to nutritional deficiencies complicating already a deficient state. Lack of libido, erectile dysfunction may be developed in these patients. Low

dopamine, serotonine etc in brain may be a common finding in these cases. Erectile dysfunction etc may be correlated to deficient steroid biosynthetic pathways in the long term of suffering phase. Vitamin E deficiency may have a role in the secondary impotency and may need therapeutic intervention.

IV. DISCUSSION

Ambiguity of genotype –phenotype relationship is reflected by cases where a single mutation affects multiple phenotype or vice versa. For example cystic fibrosis is caused by homozygous/compound heterozygous mutations in the CFTR gene & the same mutation causes congenital bilateral absence of vas-deferens (CBAVD) [36], [37], [38]. [39] Mentioned it as an example of phenotypic heterogeneity. Our observation also shows the same with Beta Thalassemia minor. Therefore an understanding of this kind of discrepancies may be considered significant from a clinical genetics point of view and small reports may not be overlooked in the age of proteomics and genomics, while proposing a therapeutic strategy. Systems biology or integrated biology which propose to virtually integrate knowledge of all domains with a single objective of human welfare and so the proposals from the NIH workshop mentioned earlier worth mentioning here again that advocate not to only rely on statistical data with large sample size, but also to evaluate results obtained in small samples and the interplay of genotype with environment. According to a *Nature* report [40] and several others [41], [42] small sample unique observations are lost in translation due to underdeveloped data analysis methods for detecting complex interaction. We are clearly heading towards personalizing therapies which will be genome-specific and in this environment unique interactions and interplay of conditions as we have reported may through light on the fact that the mystery has not yet completely resolved with the revelation of the human genome.

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