Original Research Article

Screening for Gaucher's Disease in unexplained splenomegaly and/or thrombocytopenia: An observational study

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Abstract

Introduction: Gaucher's disease is a hereditary disease that can be diagnosed by determination of acid β glucosidase enzyme activity on leucocytes but it's diagnosis is mostly delayed due to limited availability of test.To determine prevalence of Gaucher's disease in patients of unexplained splenomegaly and/or thrombocytopenia using dried blood spot filter test.**Methodology:** This prospective cross sectional study was conducted after approval from Institutional ethical committee in 222 subjects, assuming 3.6% prevalence of the disease among unexplained cases of splenomegaly with 95% confidence interval, 0.05 α error, 80% power and with an absolute allowable error of 2.5%. After implementation of the diagnostic algorithm, samples from the patients were collected on dried blood pot filter paper and sent for analysis. Patients who tested positive by screening test were confirmed through mutational analysis done from the same sample. Data was expressed as mean, proportions and percentages. Mann Whitney test,Chi square test and Fisher's exact test were used for analysis.**Results:**The prevalence of Gaucher's disease in our study population was 2.7% (CI 0.54 to 4.86) with the odds ratio for gender calculated as 3(95% CI 0.344 to 26.134).**Conclusion:**The results of this study show that the use of an appropriate diagnostic algorithm and DBS filter test facilitate early diagnosis and management of a rare disease, thereby saving a lot of medical resources while simultaneously improving the quality of life in patients.

***Key-words*:**Gaucher's disease, Dried blood spot filter test, Splenomegaly, Thrombocytopenia.

Introduction

Gaucher's disease(GD), the most common lysosomal storage disorder, is an autosomal recessive hereditary disease due to the congenital deficiency of lysosomal enzyme acid β-Glucosidase. It leads to accumulation of non-degraded glycosphingolipids (gluco cerebroside) in the macrophagic cells of the reticulo-endothelial system. The disease incidence in patients with Ashkenazi Jewish heritage varies from 1 in 450 to 1 in 1000, while in the rest of populations the incidence is estimated at 1:40000 to 1:60000.[1,2] Gaucher's disease is progressive and if not treated, it may lead to increased morbidity and mortality due to liver failure, pulmonary hypertension, skeletal complications, haemorrhage and sepsis.[3] Splenomegaly and thrombocytopenia are the two most typical and frequent manifestations of GD.[4] Though, GD can be easily diagnosed by determination of acid β glucosidase enzyme activity (<30% compared to healthy subjects) on leucocytes, taken from a peripheral blood sample, it's diagnosis is mostly delayed due to availability of test only at limited centres.[5]We hypothesised that a novel screening method of dried blood spot filter test can be used to screen Gaucher's disease in patients with unexplained splenomegaly and/or thrombocytopenia after implementation of an appropriate diagnostic algorithm. The primary aim of this study is to determine the prevalence of Gaucher's disease in patients of unexplained splenomegaly and/or thrombocytopenia using dried blood spot filter test and appropriate diagnostic algorithm. This method will eliminate the diagnostic delay, creating opportunities for the patient to receive treatment with enzyme replacement therapy(Imiglucerase) in the reversible phase of the disease and hence, reducing overall morbidity.[6]

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Materials and Methods

This cross sectional observational study was carried out in the department of pediatrics at our institute after approval from institution's ethical committee. A written and informed consent was taken from the patient's relatives after explaining the procedure to the patient. Assuming 3.6% prevalence of GD among unexplained cases of splenomegaly, sample size was calculated to be 222 with 95% confidence interval, 0.05 α error, 80% power, with an absolute allowable error of 2.5%.[7]

Inclusion criteria comprised of patients with unexplained splenomegaly and persistent thrombocytopenia(Table 1).

Exclusion criteria was defined as the cases where causes of splenomegaly and/or thrombocytopenia have been ascertained e.g. haematological malignancies, splenomegaly due to portal hypertension, haemolytic anaemia, haemoglobinopathies, acute and chronic infections.After implementation of Gaucher's disease diagnostic algorithm proposed by Mistry et al, samples from the patients were collected on Dried Blood Spot (DBS) filter paper and sent for analysis of β-glucosidase enzyme levels. The normal reference range for β-glucosidase enzyme activity, estimated by fluorometric assay on DBS samples was taken as 2.3 – 14.1 nmol/ml/hr, and patient range as 0 – 2 nmol/ml/hr.[8] Level of chitotriosidase enzyme was also measured in the test with normal range considered as <150 nmol/ml/hr. Patients who tested positive by

screening test were confirmed through mutational analysis done from the same sample.

Statistical Analysis was done with SPSS-16 and MS-Excel. Data was expressed as mean, proportions and percentages. Mann Whitney U test was used to calculate difference of means between two groups. Chi square test and Fisher exact test were used to demonstrate association between two variables, as appropriate.

Table 1: Inclusion criteria

S.no.	Inclusion criteria	
1.	Unexplained splenomegaly	Clinically palpable spleen ≥ 2 cm from the costal margin
		Splenomegaly diagnosed by Ultrasonography
2.	Persistent thrombocytopenia	Platelet count<100,000/mm^3 on 2 occasions at least 4 weeks apart alongwith bone pain or Hemogobin<10g/dl
		Platelet count<100,000/mm^3 with history of splenectomy

Results

Two hundred twenty two patients with unexplained splenomegaly and/or thrombocytopenia were enrolled in this study. Out of 222 patients, 129 patients had both splenomegaly and thrombocytopenia, 68 had only splenomegaly while 25 had only thrombocytopenia. Among the 222 patients, 6 had low(< 2 nmol/ml/hr) levels of β-glucosidase enzyme level and high chitotriosidase level (>150 nmol/ml/hr). GD patients had significant growth retardation as compared to other patients included in the study(p value- 0.005). Average Spleen size of GD patients was significantly more than other patients(p value – 0.001). Platelet count was found to be on lower side in GD patients (p value- 0.045). GD patients had higher Serum iron than other patients (p value- 0.036). On performing ultrasongraphy, GD patients had increased liver and spleen size, with p value of 0.002 and 0.016, respectively.(Table 2)

Table 2: Comparison of patient's characteristics with and without Gaucher Disease

Characteristics	Gaucher's disease present(n=6)	Gaucher's disease absent(n=216)	p value
Age	57.60±47.548	55.26±57.925	0.472
Weight For Age	13.22±7.823	14.01±10.443	0.718
Height Deficiency For Age	-2.67±0.516	-1.41±1.057	0.005
Spleen Size	11.42±3.26	5.60±3.88	0.001
USG Spleen Size	15.28±3.45	9.68±3.79	0.002
USG Liver Size	12.87±2.60	10.03±2.51	0.016
TLC	5340.00±2169.98	9207.76±7724.76	0.381
TRBC	2.67±0.67	2.82±1.08	0.794
Platelet Count	0.46±0.29	1.28±1.50	0.045
Haemoglobin	5.68±1.33	6.18±2.44	0.864
S. Iron	134.67±31.53	95.34±53.81	0.036
S. Ferritin	299.72±258.19	266.71±260.84	0.778
TIBC	260.00±22.83	283.24±78.00	0.619
SGOT	62.00±30.20	72.96±69.84	0.882
SGPT	28.67±26.74	49.79±62.418	0.079
S. Vitamin B12	370.17±113.547	370.69±239.85	0.370
Folic Acid	12.38±5.60	10.72±5.54	0.441
Beta Glucosidase Enzyme Level	1.05±0.52	5.37±4.05	0.000
Chitotriosidase Level	502.93±466.24	46.55±45.61	0.003

**Mean± SD*

Discussion

Gaucher's disease is the most common lysosomal storage disorder with the estimated prevalence of 1:60,000 to 1:40,000 in non-Ashkenazi jews population. There have been no prevalence studies in India, so the actual prevalence is not yet known. Some studies suggest overall prevalence to be 1:105, while others suggest higher number depending on the demographic profile.[9,10] As GD is a rare disease, involving multiple organs with variable clinical manifestation, it is accompanied by delayed diagnosis and delayed commencement of enzyme replacement therapy. Only twenty per-cent haematologist/oncologist considers GD in the differential diagnosis of a patient with a history of anaemia, thrombocytopenia, hepatomegaly, splenomegaly and bone pain.[11] Many a complex algorithm have been proposed for screening of patients with GD. Screening algorithm proposed by Maja et al uses five indicators i.e. skeletal erlenmeyer flask(EF) deformity, growth retardation, strabismus and/or oculomotor palsy, serum ferritin levels and tartrate resistant acid phosphatase(TRAP) levels.[12] Serum ferritin or TRAP levels are not part of routine laboratory investigations and not many

GD patients present with characteristic skeletal EF deformity, strabismus and oculomotor palsy. Therefore, relatively simple diagnostic algorithm described by Mistry et al, which uses splenomegaly and thrombocytopenia for screening was used in our study. The diagnostic algorithm proposed by Mistry et al has already been successfully applied in previous studies.[7]

The gold standard for diagnosis of Gaucher's disease is acid β-glucocerebrosidase enzyme assay in blood leucocytes but its regular use is hampered by its inaccesibilty. So, we used DBS filter test for diagnosis of GD. Though DBS filter test is not the gold standard but it has been well validated in previous studies for the diagnosis of GD.[8]The prevalence of GD in our study population i.e. patients with unexplained splenomegaly and/or thrombocytopenia, was 2.7% (CI 0.54 to 4.86) with the odds ratio for gender calculated as 3(95% CI 0.344 to 26.134). There were 5 males and 1 female. Our results were similar to the study conducted by Motta et al who found the prevalence of GD in the same patient group to be 3.6% (CI 95% 1.4–7.2) with 5 females and 2 males.[13] In our study, the mean age at presentation was 55.27 ± 5.75 months (4.6 ± 0.475 years) while the mean age of diagnosis of GD was 57.60 ± 2.26 months (4.8 ± 0.188 years). Lei et al had also observed similar findings in their study. Out of 73 children, four (three boys and one girl) were diagnosed with GD, with a median age of 1.5 years, and the prevalence in that specific population was ascertained to be 5.5% (1.5-13.4%). [8,14,15]In our study, 100% of GD cases had hepatosplenomegaly (66.67% had moderate splenomegaly and 33.33% had severe splenomegaly) and thrombocytopenia. 83.3% of patients with GD had anaemia, 66.67% had growth retardation, 33.3% had bone pain but none had radiologic bone disease. These findings were consistent with ICGG Registry as 66.67% GD cases in our study were younger than 6 year. Among the six GD patients in our study, thrombocytopenia and anaemia were observed more frequently when compared with the ICGG registration which showed 50% of children with severe or moderate platelet count, and about 40% with anaemia.[4] Genotype data from the ICGG Registry indicate that four common mutations constitute 72% of all GD alleles: N370S, L444P, IVS2+1, and 84GG.[4] Depending on the allele involved the presentation and the average age of diagnosis is highly variable. At the time of diagnosis, patients with the L444P/L444P genotype had median age of 1 year. All other allele groupings had a median diagnosis age ranging from 3 to 7 years. In our study, 50% (3) had L483P/L483P genotype, 33.33% (2) had L444P/L444P genotype and 16.67% (1) had L444P/A456P genotype. 2 out of 3 L444P genotype had neurological manifestation and 1 out of 3 L483P mutation had neurological manifestations.(Table 3)

Table 3: Comprehensive evaluation of six patients with positive DBSresult

S.no.	Chitotriosidase level	β glucosidase enzyme level	Mutation Analysis*
Patient 1	239	0.46	[L444P]+[L444P]
Patient 2	228.52	1.01	[L444P]+[A456P]
Patient 3	1051.1	1	[L444P]+[L444P]
Patient 4	789	1.63	[L483P]+[L483P]
Patient 5	1041.26	1.9	[L483P]+[L483P]
Patient 6	1232.67	0.9	[L483P]+[L483P]

**The mutations are described according to the traditional amino acid residue numbering*

The relevance of the combined approach based on Mistry et al's algorithm and the use of DBS filter test to assess the β-glucosidase enzyme activity is that it can be made easily accessible, permitting earlier diagnosis and management. Although the number of GD cases in our study was small, our findings expect paediatricians to be increasingly suspicious for diagnosis of GD in children with thrombocytopenia and/or anaemia, especially those accompanied with hepatosplenomegaly.There should be a high index of suspicion in order to diagnose rare genetic disorders in children presenting with common clinical manifestation. The diagnostic algorithm was proved to be appropriate to make an early diagnosis of GD patients with mild symptoms or atypical symptoms and avoid diagnostic delay. DBS filter test is convenient for preparation, storage and transport, and can effectively screen GD patients, hence it can be used as a preliminary screening method for GD diagnosis. These results show that the use of an appropriate diagnostic algorithm and a simple, relevant diagnostic method, such as DBS filter test, are important tools to facilitate early diagnosis and management of a rare disease, thereby saving a lot of medical resources while simultaneously improving the quality of life in patients.

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