Evaluation of CCL18 and Chitotriosidase as biomarkers for Gaucher's and Niemann Pick disease in patients from India and Pakistan

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Introduction : Gaucher's Disease is one of the lysosomal storage disorders that is caused by deficiency of enzyme β-glucosidase. The prevalence of GD is approximately 1/57,000 to 1/75,000 births worldwide[1]. In case of Indian population, series of seven cases from Malabar region in Kerala showing increased incidence in the tribal population of Mappila Muslims has been published.[2] There are 3 types of Niemann Pick diseases, viz Niemann Pick A/B and Niemann Pick C. NPA/B caused by deficiency of sphingomyelinase enzyme. NPC however shows normal enzyme activity. Both these diseases have autosomal recessive inheritance pattern.

Background : Chitotriosidase and CCL18 are proteins that are massively produced and secreted by Gaucher and NPD cells. Hence patients with Gaucher's and Niemann pick have elevated plasma levels of these two proteins. Hence it is common practice to use these proteins as biomarkers for Gaucher's as well as Niemann pick disease. In our lab we monitor plasma levels of Chitotriosidase and CCL18 of Gaucher's and Niemann Pick patients.

Objective : To evaluate suitability of CCL18 or Chitotriosidase as primary biomarker for screening of GD\NPD.

Material and Methods : This work is a retrospective study over a period of 4 years from 2011 to 2014. The patients were referred from all over India and Pakistan. Out of 150 total LSD Patients we selected 7 patients of Gaucher's and 10 patients of Niemann pick A/B and C. All of them were molecularly confirmed. Serum/Plasma samples were used for monitoring Chitotriosidase and CCL 18 levels. Chitotriosidase levels were analysed by fluorimetry using 4MU as an artificial substrate whereas CCL18 levels were detected by sandwich ELISA.

Result :

Table # 1 :- Mean levels of Chitotriosidase and CCL18

	Chitotriosidase mean ± SD	CCL18 (ng/ml)
Gaucher's Patients	5488.46 ± 7954.393	2450.68 ± 5071.27
Niemann Pick A/B Patients	173.351 ± 198.897	976.012 ± 736.360
Niemann Pick C Patients	262.05 ± 369.209	929.135± 1163.436

We found grossly elevated levels of Chitotriosidase in GD patients and elevated levels of Chitotriosidase in NPD patients. (NR: 9-46 umol/hr/ml). **2/7 Patients of GD and 2/6 NPA/B patients as well as 2/4 NPC patients showed reduced Chitotriosidase activity. CCL18 was significantly elevated in all patients** with GD and NPD. (NR: 1-72 ng/ml)

Table # 2 : Levels of Chitotriosidase & CCL18 in Gaucher's Disease

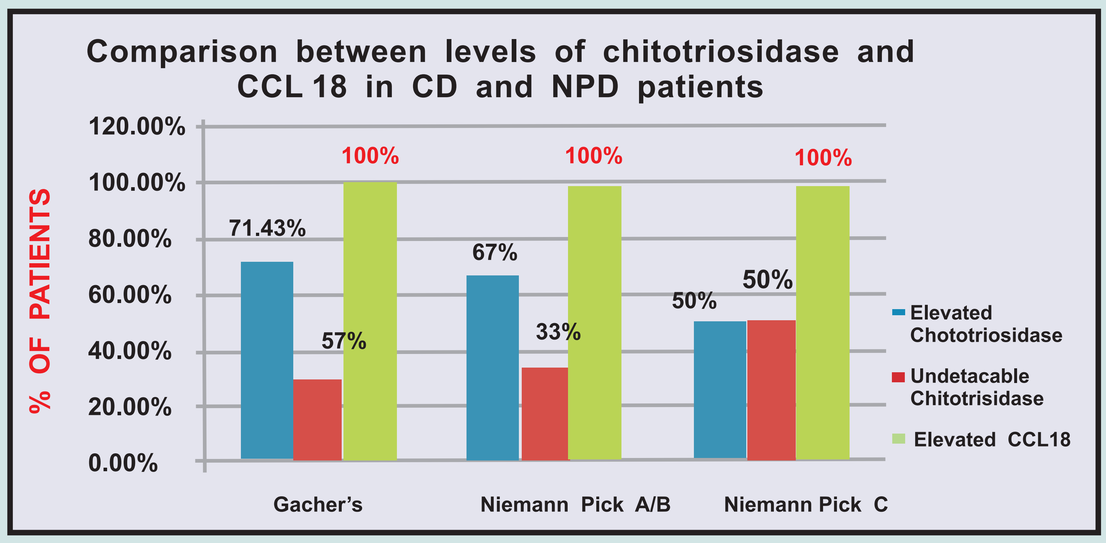
Gaucher's Patients	Chitotriosidase (umol/hr/ml)	CCL18 (ng/ml)
A	2960	1773.51
SS	22800	13882
FR	**0**	**462**
PG	**0**	**250.28**
TK	4592	266.5
F	1994	190
AS	6073.2	330.51

Table # 3 : Levels of Chitotriosidase & CCL18 in Niemann Pick C

Niemann pick patients	Chitotriosidase (umol/hr/ml)	CCL18 (ng/ml)
FB(NPC)	265	1022
HI(NPC)	**0**	**89.316**
MH(NPC)	**0**	**64.092**
A(NPC)	783.2	2541.133
FB(NPC)	265	1022

Table # 4 : Levels of Chitotriosidase & CCL18 in NPA/B patients

Niemann pick patients	Chitotriosidase (umol/hr/ml)	CCL18 (ng/ml)
HS(NPA/B)	445	112.544
MC(NPA/B)	336.3	622.68
MY(NPA/B)	**0**	**305**
QA(NPA/B)	**0**	**1833**
AM(NPA/B)	**0**	**1241.699**
P(PA/B)	258.81	1741.152



Discussion : Chitotriosidase is a chitolytic enzyme secreted by activated human macrophages as well as poly-morphonuclear leukocytes. Chitotriosidase plays an important role in host defense mechanism. Hence apart from above two diseases, Chitotriosidase may be found elevated in other pathological conditions. This suggests that it is not a specific marker for GD or NPD**. As we can see from our data around 28.57% of GD patients showed undetectable Chitotriosidase activity also 33% of NPDA/B and 50% of NPC patients showed negligible activity.** The reason is, around 5–6% of the population lacks the enzyme as a result of a genetic deficiency due to an expressional mutation in the human Chitotriosidase gene.[3] **In contrast, CCL18 was consistently elevated in all patients**. Since Chitotriosidase is an enzyme it does exhibit substrate saturation kinetics and hence it requires precise concentration of substrate to analyze its activity, which in turn limits its sensitivity, whereas **CCL18 can be monitored by using simple method like ELISA.** According to studies, Chitotriosidase does not show elevation in mildly affected Gaucher's Patients.[4] **In contrast very mildly affected individuals still show abnormal levels of CCL18.** Like Chitotriosidase, CCL18 also shows decline in concentration on onset of treatment which is an important criteria for all biomarkers.

Conclusion : Even though Chitotriosidase is the most well-established biomarker in GD and NPD, it is not specific. Furthermore, it may be falsely negative in a significant percentage of GD and NPD patients due to genetic variation. CCL18 appears to be more reliable with 100% sensitivity and therefore should be used as a primary biomarker.

Conflict of interest : None

Abbreviations : NPD- Niemann Pick Disease, GD- Gaucher's Disease, LSD - Lysosomal storage disorders, 4 -MU - 4 - Methyl4 - Methylumbelliferyl β-D-N,N′,N″-triacetylchitotrioside

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