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Differential diagnosis of mucopolysaccharidosis and oligosaccharidosis of a sample of Egyptian children

Ekram Fateen[a], Manal Fouad Ismail[b], Noha Ahmed El-Boghdady[b,*], Mona Aglan[c], Mona Ibrahim[a], Amira Radwan[a]

[a] Biochemical Genetics Department, Human Genetics and Genome Research Division, National Research Centre, Giza 12622, Egypt
[b] Department of Biochemistry, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt
[c] Clinical Genetics Department, Human Genetics and Genome Research Division, National Research Centre, Giza 12622, Egypt

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ABSTRACT

Mucopolysaccharidosis (MPS) and oligosaccharidosis are lysosomal storage disorders (LSDs) that share many clinical features. The present study aimed to establish a protocol for the biochemical diagnosis of these disorders and their subtypes in affected Egyptian children as well as in pregnant females, in order to prepare children or fetus for enzyme replacement therapy. Urine, plasma and leukocyte samples were collected from 280 children with symptoms suggestive of LSDs. Fourteen amniotic fluid samples were collected from pregnant females having positive family history or having one affected sibling. Assessment of urinary glycosaminoglycans (GAGs) followed by two dimensional electrophoresis (2-DEP), thin layer chromatographic (TLC) for separation of oligosaccharides and plasma or leukocyte enzyme activity were performed. Six of pregnancies were diagnosed to have affected fetuses. 84 children had abnormal 2-DEP and classified as 26 MPS I, 10 MPS II, 24 MPS III and 24 MPS VI. Two were diagnosed as α-mannosidosis and 2 as GM_1 gangliosidosis. In conclusion; MPS should be excluded before suspecting oligosaccharidosis. 2-DEP and TLC alone cannot rule out the diagnosis of either MPS or oligosaccharidosis and confirmation must be done by specific lysosomal enzymatic assay. Analysis of GAGs by 2-DEP in amniotic fluid can be promising method for prenatal diagnosis of MPS.

1. Introduction

Lysosomal storage disorders (LSDs) are a group of genetic disorders caused by a deficiency of specific enzymes responsible for the degradation of substances present in lysosomes [1]. Disposal of sphingolipids, glycosaminoglycans (GAGs), glycoprotein and glycogen resulting in impaired intracellular turnover.

More than 50 LSDs have been described, LSDs are generally classified by the accumulated substrates and included the sphingolipidoses, oligosaccharidoses, mucolipidoses, mucopolysaccharidoses (MPSs), lipoprotein storage disorders, lysosomal transport defects, neuronal ceroid lipofuscinoses and others [2]. Most of the patients with LSDs are born apparently healthy and the symptoms develop progressively. These symptoms may include changes in facial appearance, bone deformities, joint stiffness and pain, loss of skills such as speech and learning, respiratory and cardiac problems, behavior problems and mental retardation, sight and hearing difficulties, enlargement of the spleen and liver [3].

In MPS, GAGs as heparan sulfate (HS), dermatan sulfate (DS), chondroitin sulfate (CS) and keratan sulfate (KS) accumulate in lysosomes [4]. There are 11 known enzyme deficiency that give rise to seven distinct MPS types. MPS I (Hurler, Hurler-Scheie, Scheie) is due to deficiency of lysosomal hydrolase α-iduronidase. In MPS II (Hunter syndrome) the deficient enzyme is iduronate sulfatase. There are 4 subtypes of MPS III (Sanfilippo syndrome) (A, B, C and D) resulting from deficiency of 4 enzymes: Heparan-N-sulfatase, α-N-acetyl glucosaminidase, acetyl transferase, α-N-acetyl glucosamine-6-sulfatase. MPS IV (Morquio syndrome, A and B subtypes) is due to deficiency of 2 enzymes N-acetyl galactosamine-6-sulfatase and β-galactosidase. MPS VI (Marteaux-Lamy syndrome) is due to deficiency of N-acetyl galactosamine-4-sulfatase (aryl sulfatase B). MPS VII (Sly syndrome) is due to deficiency of β-glucuronidase. MPS IX is due to deficiency of hyaluronidase [5].

Oligosaccharidosis clinically resemble MPS but are less common. Most patients of oligosaccharidosis show more severe neurological symptoms and are more frequently symptomatic at birth.

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* Corresponding author at: Faculty of Pharmacy, Cairo University, 11562, Egypt.
E-mail address: noha.elboghdady@pharma.cu.edu.eg (N.A. El-Boghdady).

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Oligosaccharidosis include α-mannosidosis, β-mannosidosis, fucosidosis, sialidosis, galactosialidosis, aspartylglucosaminuria, depending on the defective enzyme [6]. The discovery of oligosaccharidosis in 1960 s presented the problem of its differential diagnosis from MPS [7].

Although these disorders are rare, the overall prevalence of LSDs is relatively high, compared to other groups of rare diseases, and is estimated at 1 in 5000–8000 live births [8] which makes it one of the most prevalent groups of genetic diseases in humans. The prognosis is very serious in most LSDs and great effort has always been made to find treatment options fit to face the underlying causes. A successful therapeutic approach to LSDs should ensure an available source of the deficient enzyme, thus helping the degradation of the accumulated metabolites in the various organs, and at the same time preventing their further deposition. The inherited basis implies a risk of recurrence during future pregnancies, and as prenatal diagnosis is available, most families have the opportunity to plan accordingly.

Although treatment is available for certain subtypes, early diagnosis is essential as current approaches are unlikely to restore organ function when there is considerable pre-existing pathology at the time of initiation. The present study was designed to establish a protocol for the biochemical diagnosis of MPS and oligosaharidosis and their subtypes in affected Egyptian children as well as in pregnant females in order to prepare children or fetus for enzyme replacement therapy.

2. Subjects and methods

This study included 280 children with coarse facial features, hepatospleenomegaly or mental retardation suggestive of MPS and oligosaccharidosis. They were selected from cases referred to the biochemical genetics laboratory at National Research Centre (NRC), Giza, Egypt, from April 2013 to April 2015. Children ages ranged from 1 month to 15 years.

The following were performed on cases:

- Three generations family pedigree analysis including consanguinity and other affected family members.
- Complete history development, pregnancy and delivery.
- Clinical examination of dysmorphic features and different body systems.
- Anthropometric measurements especially height, weight, head circumference to evaluate the growth parameters of the cases.
- Other investigations especially abdominal ultrasonography and skeletal survey whenever indicated.

Selected cases were divided according to their age into: Group I (1–24 months); group II (2–5 years); group III (5–10 years); group IV (10–15 years).

Fifty healthy subjects, referred as a control group with matching age and sex were included. The controls were divided into 4 groups with the same age range as that of the study groups. In addition to, 14 pregnant women with previously diagnosed children of MPS referred to the same laboratory for prenatal diagnosis were included. A written informed consent was obtained from parents of all participants and the women after full explanation of the study. The ethical approval was obtained from the medical ethical committee at the NRC and Faculty of Pharmacy, Cairo University.

Random urine and venous blood samples (~5 ml) were taken from all children. Urine samples were collected in conical plastic tube and stored untreated for the quantitative determination of total GAGs levels, two-dimensional electrophoresis (2-DEP) of GAGs and thin layer chromatography (TLC) of oligosaccharides. Blood were withdrawn on EDTA for determination of enzymatic activities in either leukocytes or plasma according to the pattern of 2-DEP and/or TLC separation. Leukocytes were separated according to the method of Cooper et al. [9].

10 ml cell-free amniotic fluid samples were withdrawn from pregnant females by amniocentesis for 2-DEP. All samples were stored at −20 °C until the biochemical analysis.

2.1. Chemicals

Substrates for enzymatic activities determination were purchased from Sigma-Aldrich Chemical Co., ST. Louis, MO, (USA). Cellulose acetate sheets of 2-DEP were obtained from Sartorius AG (Germany). All other chemicals were of highest analytical quality.

2.2. Biochemical studies

2.2.1. Determination of total urinary GAGs levels

Total GAGs levels were determined in urine samples by measuring the color formed by the reaction with dimethylmethylene blue (DMB) at 520 nm without prior precipitation of GAGs [10]. Urine creatinine concentration were determined by the method of Allen et al. [11] and expressed as mmol/dl. GAGs levels were expressed as mg GAGs/mmol creatinine.

2.2.2. Two dimensional electrophoresis of the GAGs

Qualitative analysis of GAGs by 2-DEP were performed to determine possible subtypes of MPS. In this procedure GAGs were extracted from urine or amniotic fluid, 2-DEP was carried out on cellulose acetate sheets; clear-blue spots on a white background were obtained [12].

2.2.3. Thin layer chromatography of oligosaccharides

Urine samples corresponding to 20 μg creatinine were applied on silica gel TLC plate. The plate was developed twice in a freshly prepared mixture of n-butanol: glacial acetic acid: distilled water (100:50:50, v/v/v). The plate was dried and sprayed with a freshly prepared solution of orcinol (0.2 g/dl) in sulfuric acid. The oligosaccharide bands were visualized by heating [9].

2.2.4. Fluorometric enzymatic assay of mucopolysaccharidosis

Fluorometric enzyme assays were done according to the abnormal 2-DEP pattern to confirm the type of MPS. The activity of the enzymes was measured using the artificial substrates: 4-methylumbelliferyl-α-L-iduronide, 4-methylumbelliferyl-α-L-iduronate-2-sulfate, 4-methylumbelliferyl sulfate and 4-methylumbelliferyl-*N*-acetyl-α-D-glucosaminide as substrates for the determination of α-iduronidase, iduronate-2-sulfatase, arylsulfatase B and α-*N*-acetyl glucosaminidase, respectively. The enzyme cleaves the acid from the substrate to yield the fluorescent product, which is then measured using a fluorescent spectrometer against a standard of 4-methylumbelliferone [13–16].

2.2.5. Fluorometric enzymatic assay of oligosaccharidosis

Fluorometric enzyme assays were done according to the abnormal TLC pattern to confirm the type of oligosaccharidosis using the artificial substrates: 4-methylumbelliferyl-α-fucopyranoside, 4-methylumbelliferyl-β-D-galactopyranoside, 4-methylumbelliferyl-β-D-mannoside and 4-methylumbelliferyl α-D-mannopyranoside as substrates for the determination of α-L-fucosidase, β-galactosidase, β-mannosidase and α-mannosidase, respectively [9,17–19].

2.3. Statistical analysis

Data were computed as means ± standard deviation (SD). Statistical analysis were conducted using SPSS (Statistical Package for Social Sciences) version 10 and Microsoft excel 2006. Means and SDs of means were calculated and statistical significance was tested by *t*-test. The level of significance was set at $p < 0.05$.

3. Results

3.1. Clinical characteristics

The age distribution was 3.5 ± 3.1, 5 ± 4.4 and 31.6 ± 2.5 years in the 280 cases, the controls and the 14 pregnant females, respectively. The 280 studied children included 170 males (60.7%) and 110 females (39.3%) while the 50 control subjects included 27 males (54%) and 23 females (46%). The overall consanguinity rate recorded in the current study was 63.6% (178 cases) among the 280 studied children, 58% (29 cases) among the controls and 11 of the 14 pregnant females had positive consanguinity. On the other hand, 216 cases (77.14%) had no family history of the disease while 50 cases (17.85%) had undiagnosed siblings and 14 cases (5%) had previously diagnosed siblings with MPS.

Different clinical manifestations and their frequency of distribution were recorded among the studied cases. The most commonly detected clinical manifestations were short stature (66%) followed by macrocephaly (56%), coarse facial features (53%), hepatospleenomegaly (51.4%) and dysostosis multiplex (37.5%).

3.2. Biochemical findings

3.2.1. Level of total urinary GAGs

Table 1 shows the levels of urinary GAGs in the 280 cases and the controls. As urinary GAGs excretion decreases with increasing age, results were compared with the age matched controls. Normal level of urinary GAGs were found in 46 (16.43%) and significantly high in 234 (83.57%) children.

3.2.2. 2-DEP separation of urinary GAGs

Table 2 shows the classification of the 280 cases according to their electrophoretic pattern. The 46 cases with normal urinary GAGs excretion revealed normal electrophoretic pattern. The normal electrophoretic pattern consists of a single CS spot but a slight H, HS spot can be observed in infants below 1 year of age. Among the 234 cases with high urinary GAGs excretion, there were 150 cases with normal electrophoretic pattern; 36 cases with spots of CS, DS, H, HS which is suggestive of MPS type I, II or VII; 24 cases showed spots of CS, H and HS which is suggestive of MPS III and 24 cases with spots of CS and DS which is suggestive of MPS VI. Regarding the pregnant females 8 cases revealed normal pattern while the other 6 cases revealed abnormal pattern.

3.2.3. Assessment of enzyme activities to confirm diagnosis of MPS

Enzyme activity was measured for children according to the abnormal 2-DEP pattern of MPS. Table 3 shows that, in the 36 cases suggestive as MPS type I, II or VII α-iduronidase enzyme was deficient in 26 cases and diagnosed as MPS type I and iduronate-2-sulfatase enzyme was deficient in 10 cases and diagnosed as MPS type II. In the 24 cases suggestive as MPS type III, N-acetyl glucosaminidase enzyme was deficient in 11 cases and diagnosed as MPS IIIB and the other 13 cases were considered as MPS III A, C or D. In the 24 cases suggestive as MPS VI, arylsulfatase B enzyme was deficient in all the 24 cases.

Table 1
Urinary GAGs levels in different age groups of studied children and controls.

Group	Age	Control group n = 50	Cases n = 280	
			Normal GAGs n = 46	High GAGs n = 234
I	1–24 m	25.97 ± 5.76 (n = 20)	27.93 ± 12.22 (n = 17)	58.45 ± 40.62* (n = 103)
II	2–5 y	15.69 ± 1.97 (n = 17)	17.75 ± 9.60 (n = 11)	46.20 ± 27.28* (n = 97)
III	5–10 y	12.52 ± 1.08 (n = 9)	16.70 ± 14.26 (n = 10)	30.30 ± 13.01* (n = 25)
IV	10–15 y	9.63 ± 0.90 (n = 4)	12.49 ± 7.01 (n = 8)	25.02 ± 6.85* (n = 9)

Data are expressed as Mean ± SD, n = number of children, *p < 0.05 against control values.

Table 2
Distribution of children and pregnant females according to their 2-DEP pattern and urinary GAGs.

2-DEP pattern		Pregnant females (n = 14)	Children (n = 280)	Urinary GAGs
Normal pattern		8 (57.2%)	196 (70%)	Normal (n = 46) High (n = 150)
Abnormal pattern	CS, DS, H and HS (MPS I & II)	3 (21.4%)	36 (12.8%)	High (n = 84)
	CS, DS (MPS VI)	2 (14.3%)	24 (8.5%)	
	CS, H and HS (MPS III)	1 (7.1%)	24 (8.5%)	

DS: dermatan sulfate, H: heparan, HS: heparan sulfate, CS: chondroitin sulfate, n = number of cases.

Table 3
Activities of some lysosomal enzyme in children with MPS and controls.

Enzyme	Children	Controls	Normal range
α-Iduronidase (nmol/mg protein/h)	0.46 ± 0.36* (n = 26)	27.66 ± 3.1 (n = 21)	10–40 [13]
Iduronate-2-sulfatase (nmol/mg protein/4h)	0.15 ± 0.07 * (n = 10)	10.78 ± 1.36 (n = 10)	7.5–13.3 [14]
N-acetyl glucosaminidase (nmol/ml /h)	0.71 ± 0.41* (n = 11)	28.55 ± 3.78 (n = 11)	10–45 [16]
Arylsulfatase B (nmol/mg protein/h)	3.35 ± 2.9* (n = 24)	200.55 ± 12.2 (n = 21)	100–250 [15]

Data are expressed as Mean ± SD, n = number of cases, *p < 0.05 against control values.

3.2.4. TLC separation of oligosaccharides

TLC was performed for the 46 cases that had normal GAGs levels and normal 2-DEP pattern. Table 4 shows that 30 cases (65.2%) recorded normal pattern of oligosaccharides and 16 subjects revealed an abnormal oligosaccharides TLC profile. Among the 16 abnormal oligosaccharidosis 11 (23.9%) cases were suggestive as α-mannosidosis; 1 case (2.1%) as β-mannosidosis; 1 case as α-fucosidosis and 3 cases (6.5%) as GM1 gangliosidosis.

3.2.5. Assessment of enzyme activities to confirm diagnosis of oligosaccharidosis

Enzyme activity was measured for patients according to the abnormal TLC pattern of oligosaccharides. Table 5 shows that α-mannosidase enzyme was deficient in 2 out of 11 cases suggestive of α-mannosidosis cases. Similarly, β-galactosidase enzyme was deficient in 2 out of 3 cases suggestive of GM1 gangliosidosis cases. While β-mannosidase enzyme and α-fucosidase enzymes was not deficient in neither case who had TLC pattern suggestive of β-mannosidosis or α-

Table 4
Distribution of children according to their TLC pattern.

Thin layer chromatographic pattern		Number of children	%
Normal pattern		30	65.2
Abnormal pattern	Pattern of α-mannosidosis	11	23.9
	Pattern of β-mannosidosis	1	2.1
	Pattern of α-fucosidosis	1	2.1
	Pattern of GM1 gangliosidosis	3	6.5

Data are expressed as number and percentage of 46 cases.

Table 5
Activities of some lysosomal enzyme in children with oligosaccharidosis and controls.

Enzyme	Children	Controls	Normal range
α-Mannosidase nmol/ml/h	22.1 ± 16.8* (n = 2)	305 ± 14.1 (n = 2)	100–367 [19]
β-Galactosidase nmol/mg protein/h	8.15 ± 0.7* (n = 2)	202.75 ± 10.9 (n = 2)	100–400 [18]
β-Mannosidase nmol/ml/h	600 (n = 1)	590 ± 28.3 (n = 2)	240–800 [9]
α-Fucosidase nmol/ml/h	172 (n = 1)	112.9 ± 14.7 (n = 2)	24–292 [17]

Data are expressed as Mean ± SD, n = number of cases, *p < 0.05 against control values.

fucosidosis.

4. Discussion

Mutational defects in genes encoding enzymes that are involved in the lysosomal degradation of oligosaccharide chains in glycoproteins and of mucopolysaccharide chains in proteoglycans lead to chronic and progressive storage disorders that have many common clinical features. Diagnosis of these disorders is initially made by detecting partially degraded oligosaccharides and GAGs in urine of affected children and is confirmed by measuring the specific enzyme activity in plasma or leukocytes [20].

In the present study, 88 children (31.42%) were diagnosed from total 280 cases; 84 (95.5%) as MPS and 4 (4.5%) as oligosaccharidosis. This was in line with Piraud et al. [4] who diagnosed 199 (9.95%) cases from total 2000 cases; 170 (85.5%) with MPS and 29 (14.5%) with oligosaccharidosis. The remaining 192 cases which are not diagnosed might have other disorder with similar symptoms such as sialidosis, mucolipidosis II or III, galactosialidosis and aspartylglucosaminuria. The most common MPS type detected in the present study was MPS type I which was detected in about 30% of the diagnosed cases. While the Egyptian study of Shawky et al. [21] found MPS III was the most common type (35%) which represents about 27% of our diagnosed cases. The true distribution of MPS among the Egyptian population will become known only when progress in therapy will make it desirable to introduce early screening. On the other hand, the least common MPS type detected in the present study was MPS type II which was detected in about 11% of the diagnosed cases. While in the Italian study of Di Natale et al. [22] MPS II was the most common type and was present in about 50% of the diagnosed cases. This difference might be due to racial difference between the two countries. The oligosaccharidosis cases diagnosed in the present study were; 2 with α-mannosidosis and 2 with GM1 gangliosidoses, while Piraud et al. [4] oligosaccharidosis cases were only 7 with GM1 gangliosidoses.

Parental consanguinity was 63.6% among our study group, and reached up to 80.6% in the 88 diagnosed children. This was consistent with many previous investigators [21,23–24] who showed that parental consanguinity exceeded 80%; 75% and 89.9% in diagnosed cases. The high frequency of consanguineous marriages among Egyptian patients can be attributed to socio-cultural factors, such as maintenance of family structure and property, ease of marital arrangements, better relations with in-laws, and financial advantages relating to dowry.

The male predominance (60.7%) in the present work was obvious and agreed with the work of Fateen et al. [25] and this might be due to the presence of 10 cases with MPS II which is an x-linked disorder and the oriental culture which cares more for boys and gives them special attention.

Most of the clinical manifestations of MPS and oligosaccharidosis appears in the first 2 years of life [26] and this agrees with the present study group since more than 40% of referred children were in the first group (1–24 months). In addition, coarse facial features, organomegaly and mental retardation were found in 79 (90%), 67 (76.2%) and 60 (68.2%) respectively, of the 88 diagnosed cases. These findings are in accordance with that of Neufeld and Muenzer [26] that reported mental retardation as a characteristic feature of MPS types I, II and III but normal intelligence may be retained in other types.

Monodimensional electrophoretic method is less tedious than the bidimensional one for testing large number of samples[4]. While, 2-DEP was used in the present study for its advantages to allow better separation of each type of GAGs and it allows the coelectrophoresis of GAGs samples with its standard or control sample.

The decreased urinary GAGs levels in relation to age in our study and control group agrees with previous studies that demonstrated children excrete more GAGs during the first few months after birth which then declines by age [25,27]. Manley et al. [28] attributed the high urinary excretion of GAGs to rapid skeletal growth in infancy. However Schachtschabel and Wever [29] refereed the decrease in the excretion of GAGs with age to the gradual decline in their synthesis. Various clinical conditions can cause slight abnormalities of urinary GAGs excretion like; various bone diseases, connective tissue diseases, hypothyroidism, urinary dysfunction and aspartylglucosaminuria [4]. This may explain the presence of 150 cases out of the 234 cases with high GAGs levels and had normal pattern of 2-DEP. So, quantitative measurement of GAGs alone cannot be diagnostic for MPS as it could be increased in other conditions. On the other hand, Tomatsu et al. [30] reported high GAGs excretion in some cases with mucolipidosis and α-fucosidosis, as we didn't diagnose any case with α-fucosidosis these cases may have been missed because we performed TLC only for cases with normal GAGs level and 2-DEP pattern.

In the present study 11 cases had pattern suggestive of α-mannosidosis but only 2 of them were confirmed by their low activity of α-mannosidase enzyme. These 9 cases confused with the 2 diagnosed cases may be explained by what's known as "neonatal excretion patterns" due to milk oligosaccharide excretion. The same "neonatal pattern" can be observed in the urine of pregnant or lactating women and in patients receiving dextran infusions or plasma expanders and are not disease specific [6].

As the electrophoretic pattern does not allow MPS III subtypes to be distinguished, nor differentiated MPS II from MPS I patients [4]. Likewise, the TLC of oligosaccharides method is suitable as an initial screen to identify or exclude patients suffering oligosaccharidosis. As in glycogen storage disease type II, III and VI patients could excrete a characteristic oligo or tetrasaccharide [31–33]. So, final diagnosis must be confirmed by specific enzyme analyses.

All these disorders are inherited in autosomal recessive manner except MPS II which is X-linked disorder, which means a probability of 25% from each pregnancy to have an affected child. These affected children are burden on the whole society. The present study tried to help such families from repeating such painful experience by offering prenatal diagnosis for 14 pregnant females through 2-DEP for amniotic fluid withdrawn by amniocentesis at 15–18 weeks of gestation which revealed 6 affected fetuses and 8 normal fetuses. The prenatal diagnosis gives the choice to the family to terminate the pregnancy or early intervention with enzyme replacement therapy after the delivery.

In conclusion, differential diagnosis of children having suggestive symptoms of MPS and oligosaccharidosis is achieved by 2 sequential steps. The quantitative and qualitative (2-DEP) detection of GAGs in the urine to diagnose or exclude the presence of MPS and TLC for oligosaccharides analysis to determine the possibility of oligosaccharidosis. Then, followed by enzymatic assay for the suspicious type to confirm the diagnosis. 2-DEP and TLC are used only as screening methods not as diagnostic method and confirmation must be done by enzymatic assay. All types of MPS could be diagnosed prenatally. Analysis of GAGs by 2-DEP in amniotic fluid is a useful method for prenatal diagnosis of MPS.

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Conflict of interest

None.

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