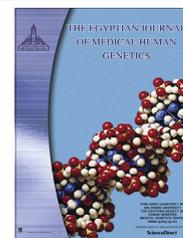




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ORIGINAL ARTICLE

# Prenatal genetic testing, counseling and follow-up of 33 Egyptian pregnant females with history of mucopolysaccharidoses



Khaled R. Gaber <sup>b</sup>, Mona M. Ibrahim <sup>a,\*</sup>, Mona K. Farag <sup>b</sup>, Zeinab Y. Abdallah <sup>a</sup>, Sara H. Eldessouky <sup>b</sup>, Ekram M. Fateen <sup>a</sup>

<sup>a</sup> Biochemical Genetics Department, Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt

<sup>b</sup> Prenatal Diagnosis Department, Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt

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## KEYWORDS

Mucopolysaccharidosis;  
Alpha-fetoprotein;  
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**Abstract** *Background:* Mucopolysaccharidoses (MPS) are autosomal recessive disorders characterized by deficiency of lysosomal enzymes which break down the glycosaminoglycans (GAGs) which results in widespread intra and extra-cellular accumulations of GAGs. Early initiation of treatment, before the onset of irreversible tissue damage, clearly provides a favorable disease outcome. Early detection might be afforded by analysis of amniotic fluid.

*Aim:* To report our experience of prenatal diagnosis of MPS over 14-year period for cases referred from medical centers throughout Egypt. Also to report the benefit of prenatal genetic testing in cases accompanied with genetic disorders.

*Materials and methods:* The present study included 33 pregnant women at risk of having a fetus with MPS. Of these cases, 3 women had more than one pregnancy evaluated. All cases had a detailed genetic ultrasound examination and a maternal serum alpha-fetoprotein (MSAFP) evaluation during the second trimester of pregnancy. Thirty-eight amniocentesis procedures were performed during the study for 2 dimensional electrophoresis (2-DEP) of GAGs.

*Results:* Positive consanguinity was present in near 70% (23/33) of the couples. Detailed genetic ultrasound examination revealed a case with anencephaly and another one with a twin pregnancy.

\* Corresponding author at: National Research Centre, Tahrir st., Dokki, Giza, Egypt. Tel.: +20 01001370926; fax: +20 0237601877. E-mail addresses: [krgeber@gmail.com](mailto:krgeber@gmail.com) (K.R. Gaber), [monamahmoud60@yahoo.com](mailto:monamahmoud60@yahoo.com) (M.M. Ibrahim), [monakamal262@hotmail.com](mailto:monakamal262@hotmail.com) (M.K. Farag), [zeinabwakad@yahoo.com](mailto:zeinabwakad@yahoo.com) (Z.Y. Abdallah), [saraeldessouky@yahoo.com](mailto:saraeldessouky@yahoo.com) (S.H. Eldessouky), [efateen@yahoo.com](mailto:efateen@yahoo.com) (E.M. Fateen).

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One case had a MSAFP of 3.6 multiple of the normal median (open neural tube defect). Another 2 cases had a risk of having Down syndrome. Results of the 2-DEP of GAGs in amniotic fluid revealed 36.8% (14/33) affected fetuses. During the final counseling setting of the 14 cases with abnormal results, 43% (6/14) elected to continue their pregnancy while 57% (8/14) elected termination.

*Conclusion:* Early prenatal screening and diagnosis, through a systematic multidisciplinary approach, to all cases of mucopolysaccharidoses are recommended, to improve the quality of life and to avoid the presence of other associated fetal developmental malformations.

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## 1. Introduction

Mucopolysaccharidoses (MPS) are autosomal recessive disorders characterized by deficiency of lysosomal enzymes which break down the glycosaminoglycans (GAGs). Their deficiency results in widespread intra and extra-cellular accumulations of GAGs [1]. They have been subdivided according to enzyme defect and systemic manifestations [2].

Together with the lipidoses, MPS are the most common lysosomal storage diseases. The overall incidence of each group is in the region of 1:10.000 births [3].

Early initiation of treatment, before the onset of irreversible tissue damage, clearly provides a favorable disease outcome. However, early diagnosis is difficult due to the rarity of these disorders in combination with the wide variety of clinical symptoms. Thus, much interest exists in prenatal detection by amniocentesis. Early detection might be afforded by analysis of amniotic fluid because it contains fetal urine and cells which commensurate with fetal age [4].

Prenatal genetic testing occurs in clinical settings, usually as part of obstetric care. The prenatal screening is the first step toward prenatal diagnosis of congenital malformations [5]. Prenatal genetic testing has initiated a great debate about reporting findings identified by a screening test to a population booked for a specific diagnostic test [6]. However, the primary purpose of prenatal testing is to gain information about the health of a fetus that may be at increased risk for a chromosomal or other genetic condition [7]. This is in addition to relieving parents' anxiety over inheriting a genetic disease or giving birth to a child with congenital abnormalities and this is the major outcome [6,7].

The purpose of this study is to report our experience of prenatal diagnosis of mucopolysaccharidosis over 14-year period (2000–2014) for cases referred from medical centers throughout Egypt. Also we will emphasize on the importance and role of prenatal genetic testing in all pregnancies, in screening for accompanied genetic disorders, even in cases where prenatal diagnosis is tailored to a specific disorder.

## 2. Patients and methods

### 2.1. Patients

This retrospective study was conducted at the Prenatal Diagnosis and Fetal Medicine Department and Biochemical Genetics Department, National Research Centre, Egypt, over a period of 14 years (2000–2014).

The study included 36 pregnancies (14–22 weeks gestation) in 33 families where a diagnosis with one of the MPS types has been made in one or more of their previous children. The exclusion criteria included previous cases with MPS IV.

The work was carried in accordance with the code of ethics of the world association (declaration of Helsinki) for experiments involving humans. A written informed consent was obtained from all pregnant women included in this study after full explanation of the study. The ethical approval was obtained from the medical ethics committee at the National Research Centre.

### 2.2. Methods

All cases in this study were subjected to:

- History taking, pedigree construction and clinical examination.
- Two counseling settings: The first entailed detailed explanation of the condition to be studied and the investigations to be carried. The second one included detailed explanation of the results and options to be carried by the parents.
- Detailed genetic ultrasound examination at time of presentation during the second trimester. This was done for detection of multiple pregnancies, placental location, fetal growth, soft tissue markers and the presence of associated fetal abnormalities.
- Measurement of maternal serum alpha-fetoprotein (MSAFP): 5 cc venous blood was collected during the second trimester of pregnancy (15 weeks<sup>+0</sup>–18 weeks<sup>+6</sup>). It was measured in duplicate using commercially available enzyme immunometric assay kit (EIA) Can Ag Diagnostics, Sweden. The values were transformed to multiple of the normal median (MoM) after adjusting the gestational age. A MSAFP above 2.5 MoM (cutoff limit) was considered elevated and carries a high risk for open neural tube defects, and the case was offered a second detailed ultrasound examination, if not detected before. MSAFP MoM was evaluated according to gestational age and older maternal age at the expected time of delivery for risk of Down syndrome. A risk of 1:270 or more was considered elevated, and the case was offered the opportunity for amniotic fluid study for fetal chromosomes.
- Extraction of GAGs from cell-free amniotic fluid: The amniotic fluid samples were centrifuged and the supernatants were taken and used for the extraction of GAGs by formation of complexes with alcian blue 8GX. In this procedure 3 ml of centrifuged amniotic fluid was mixed with

alcian blue reagent containing 50 mmol MgCl<sub>2</sub>. Blue complex was formed and after centrifugation it was separated and precipitated. The complex was dissociated by shaking with 4 mol/l NaCl and methanol. Free alcian blue was precipitated by the addition of 0.1 mol/l Na<sub>2</sub>CO<sub>3</sub> and water. GAGs were precipitated from clear supernatant by the addition of ethanol and the precipitate after centrifugation was taken up in 20 µl water [8,9].

- Two-dimensional electrophoresis: After extraction of GAGs from the amniotic fluid, 2-DEP was carried out on cellulose acetate sheets. Two microliters of GAGs were applied and the separation proceeded for 1 h as first run (pyridine:glacial acetic acid:water; 10:1:89 v/v) and then for 3 h as second run (0.1 M barium acetate buffer). The GAGs were separated into discrete spots and were easily located after staining for 1 h with 0.05% alcian blue solution containing 50 mmol/l MgCl<sub>2</sub> in 50 mmol/l sodium acetate buffer (pH 5.8) and washing with 5% acetic acid solution. Two spots should be present in any 2-DEP of amniotic fluid before further interpretation. The first is the hyaluronic acid spot verifying its amniotic fluid nature and the second is the small chondroitin spot to verify the presence of fetal urine indicating functioning fetal kidneys [9,10].

### 3. Results

The studied cases were referred from all over Egypt for prenatal diagnosis of MPS. Positive consanguinity was present in near 70% (23/33) of the couples. The maternal age at time of delivery ranged between 22 and 38 years. The gestational age at time of the procedures ranged between 15<sup>+0</sup> and 19<sup>+0</sup> weeks.

Of these cases, 3 women had more than one pregnancy evaluated during the course of the study, making them 37 pregnancies evaluated and counseled. All cases had a detailed genetic ultrasound examination which revealed a case with anencephaly and another one with a twin pregnancy (Dichorionic-Diamniotic). All cases had a MSAFP evaluation during the second trimester of pregnancy. Of these cases, one case had a MSAFP of 3.6 MoM and confirmed by ultrasound to have an open neural tube defect (anencephaly). Another 2 cases had a risk of having Down syndrome of more than 1:270 based on MSAFP measurement and the maternal age at the expected time of delivery.

Thirty-eight amniocentesis procedures were performed during the study (2 separate samples in the twin pregnancy). For the 38 amniotic fluid samples, analysis was performed in all of them for the type of MPS at risk. And, in the 2 samples

at additional risk for Down syndrome, amniotic fluid study revealed normal karyotypes. During the final counseling settings of the 14 cases with abnormal results, 43% (6/14) elected to continue their pregnancy and 57% (8/14) elected termination.

Results of 2-DEP of GAGs in amniotic fluid revealed 36.8% (14/38) affected fetuses with MPS and the distribution of the types among them is shown in Table 1. Examples of the abnormal electrophoresis patterns for MPS VI compared with the normal pattern are illustrated in Fig. 1.

### 4. Discussion

Consanguineous marriages are frequent among Egyptians and lead to high incidence of recessive genetic disorders. In the present study, positive consanguinity was detected in 70% (23/33) of the couples. The study performed by Temtamy et al. in 2010 stated that the rate of consanguineous marriages in Egypt may reach up to 40% [11]. The high rate of consanguinity in our study agrees with the rate reported (87.5%) among the pregnancies subjected to prenatal diagnosis of lipiodosis in the same community [12].

MPS can be diagnosed prenatally by measuring the enzyme activity in culture for amniocytes or in chorionic villi and also in leukocytes and plasma of fetal blood obtained by cordocentesis. It can also be achieved by the analysis of pertinent metabolites in amniotic fluid through 2-DEP of GAGs [13]. Analysis of cell-free amniotic fluid offers the advantage of being a rapid and reliable method, and eliminating the requirement for culture for amniocytes. There is a general agreement that fetal urine is a major contributor to the formation of amniotic fluid from mid-pregnancy [14].

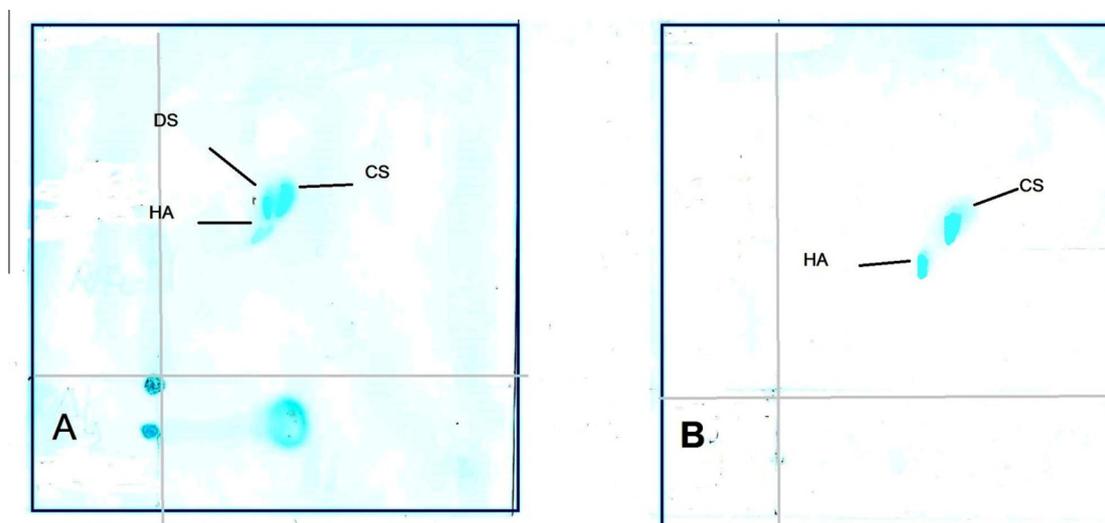
In this study we did not include any case for prenatal diagnosis of MPS IV, as we will get a normal electrophoretic pattern with spots of chondroitin sulfate and hyaluronic acid and not a spot of keratan sulfate which is the diagnostic marker for MPS IV in electrophoresis. Burlingame et al. in 1981 stated that keratan sulfate is probably bound to or associated with a certain amount of chondroitin sulfate as judged by its electrophoretic mobility before and after the use of enzymatic digestion by chondroitinase ABC [15,16].

MPS I (Hurler syndrome) results from deficiency of the enzyme alpha-L-iduronidase (IDUA). The 2-DEP of GAGs in the amniotic fluid of an affected fetus with MPS I shows excretion of dermatan sulfate and heparan sulfate, in addition to excretion of chondroitin sulfate and hyaluronic acid which are normally present in the amniotic fluid. In our study 13 pregnancies were scheduled for prenatal diagnosis of MPS I. One of the women had a previous history of affected twins and then she was tested in two successive pregnancies which

**Table 1** Results of amniotic fluid biochemical studies in pregnancies at risk of having MPS fetuses and the distribution of types of MPS among the 14 affected pregnancies.

Pregnancies at risk of having different types of MPS	Number of affected pregnancies with each type of MPS	Results of amniocentesis
13 pregnancies at risk of having MPS I fetus	5 fetuses with MPS I (35.7%)	DS, HS, CS, HA
7 pregnancies at risk of having MPS II fetus	4 fetuses with MPS II (28.6%)	DS, HS, CS, HA
9 pregnancies at risk of having MPS VI fetus	4 fetuses with MPS VI (28.6%)	DS, CS, HA
7 pregnancies at risk of having MPS III fetus	1 fetus with MPS III (7.1%)	Hep, HS, CS, HA

CS = chondroitin sulfate; HS = heparan sulfate; DS = dermatan sulfate; Hep = heparin; HA = hyaluronic acid.



**Figure 1** Example of abnormal pattern of electrophoretic separation of GAGs in amniotic fluid in MPS VI fetus (A) compared with a normal pattern (B): CS = chondroitin sulfate, DS = dermatan sulfate, HA = hyaluronic acid.

unfortunately were also affected. Another woman had history of 2 affected children (one of them died) and then she was tested in three successive pregnancies. The fetus tested in 2009 was normal, while the other 2 pregnancies were in 2012 and 2013 and resulted in 2 affected fetuses. This highlights the importance of 2-DEP of amniotic fluid as a rapid, sensitive and reliable method for the diagnosis of MPS. Another woman had a history of previously affected children and scheduled for prenatal diagnosis and the result was a normal fetus. One of the women had an affected child and was tested in 2 successive pregnancies, the result was one normal fetus and one affected. In the other 4 females (each of them had a previously affected child) the electrophoretic patterns were normal in 3 of them while one fetus was affected.

MPS II (Hunter) is an X-linked disorder caused by deficiency of iduronate-2-sulfatase (IDS) enzyme. In this study 7 pregnancies were tested for prenatal diagnosis of MPS II and 3 fetuses were proved to be affected. In one of the 3 affected pregnancies the woman had previously 3 affected boys and in the culture of Egypt, to have a normal boy is a strong motive to be pregnant several times. In the other 2 affected pregnancies, each one had only one affected child. For the other 4 normal fetuses, the women had a history of a previously affected child. One of the 4 cases with MPS II had anencephaly with elevated levels of MSAFP.

MPS type III (Sanfilippo) results from deficiency in 4 different enzymes of heparan metabolism giving subtypes A, B, C and D which are similar phenotypically and they have also similar electrophoretic pattern of GAGs separation [17]. Electrophoresis can discriminate between broad classes of MPS but cannot distinguish subgroups. Seven pregnant females were scheduled for prenatal diagnosis of MPS III. One of them had abnormal pattern with increased excretion of heparan sulfate and had a history of a previously affected child while the other 6 had normal patterns. Four out of the 6 normal pregnancies had a history of one affected child. In the other 2 normal fetuses, one had a history of previously affected twins and the other one had one normal and two affected children.

In MPS VI (Maroteaux–Lamy Syndrome), the deficiency is in arylsulfatase B enzyme (N-acetylgalactosamine-4-sulfatase) which leads to accumulation of dermatan sulfate in different tissues [18]. Nine cases in the present study were scheduled for prenatal diagnosis of MPS VI and 2-DEP of GAGS revealed 4 affected fetuses. All of the 9 cases had a history of a previously affected child with MPS VI. Fig. 1 shows the electrophoretic pattern of one of the 4 MPS VI fetuses included in this study with dermatan sulfate spot in addition to chondroitin sulfate and hyaluronic acid spots which are normally excreted in the amniotic fluid.

Prenatal diagnostic techniques have rendered decision making about reproduction more complicated for the obstetrician than any preceding era. Prenatal genetic testing, including both screening and diagnostic procedures, has a clear effect on the experience of pregnancy for many women. All pregnancies included in the study had screening tests although the main indication for referral, to the Prenatal Diagnosis Clinics–NRC, was a history of a previously affected child with MPS in the family. This included a detailed genetic ultrasound examination and MSAFP measurement during the second trimester. All cases at risk elected to perform diagnostic tests, in order to obtain indicative results concerning their fetuses.

Pregnancies at risk for Down syndrome suspected by biochemical screening (MSAFP) and older maternal age had amniotic fluid analysis for MPS I and MPS VI in addition to chromosomal study. All results were normal and the cases continued their pregnancies with normal outcome. In these mentioned cases, the primary purpose of testing was to relieve anxiety of parents over inheriting a genetic disease and to avoid the birth of a baby with an unexpected congenital abnormality other than that indicated for referral.

In one of the cases presenting with history of MPS II, the MSAFP showed increased level (3.6 MoM), and ultrasound examination revealed an open neural tube defect (anencephalic fetus). The case requested to perform amniocentesis to confirm the cause of referral. The 2-DEP of amniotic fluid revealed an affected fetus with MPS II. Closed cephalocele is a new,

peculiar and likely overlooked neuroradiologic feature of patients affected by Hunter syndrome. Manara et al. in 2011 reported that nearly 1 out of 4 Hunter patients presents with a meningeal or brain parenchymal herniation at the level of the anterior or middle cranial fossa [19].

Counseling before and after prenatal genetic testing is crucial. In an ideal counseling, the patient should be told that there is no “right” decision to be made, and that they are supported in whatever decision they make [20]. In this study, the type of the test offered either screening or diagnostic, was clearly explained. In addition, the degree of accuracy of the test in their particular situation was clearly stated. Following delivery of the results, 57% (8/14) of the couples with affected cases elected termination of pregnancy, while the remaining chose continuation. For all couples who elected to continue their pregnancies, with an abnormal result, there was false religious and social pressure about termination. This, in addition to the feeling of guilt experienced by many couples for termination of pregnancy.

In conclusion, mucopolysaccharidoses which are multisystem disorders, present with a constellation of clinical findings. Early prenatal screening and diagnosis to all cases are recommended through a systematic multidisciplinary approach. This is to improve the quality of life and to avoid the presence of other associated fetal developmental malformations. Qualitative determination of glycosaminoglycans in amniotic fluid using the 2-DEF is proved to be a rapid, sensitive and accurate method, but requires care in application and experience in interpretation. It helps couples to have a normal infant and prevent having another affected child.

#### Conflict of interest

The authors declare no conflict of interest.

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