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# Mass spectrometry and two-dimensional electrophoresis in prenatal diagnosis of mucopolysaccharidosis type VI

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## Abstract

**Background:** Mucopolysaccharidosis VI (MPS VI) or Maroteaux–Lamy syndrome is an autosomal recessive lysosomal storage disorder. Clinical manifestations are related to progressive accumulation of dermatan sulfate (DS). Two-dimensional electrophoresis has traditionally been used for the diagnosis of MPS disorders. The method is only qualitative and is time consuming. For prenatal diagnosis of MPS, 6–8 ml of amniotic fluid is required and 5 working days to complete. It needs personal experience to do the test and to interpret the results. Mass spectrometry (MS) is now available as a quantitative method and for prenatal diagnosis of MPS it needs less amniotic fluid and takes only 2 working days. It is more accurate, less person dependent, but it costs more. Our aim was to introduce quantitative determination of dermatan sulfate using mass spectrometry in the prenatal diagnosis of MPS VI in Egypt and to compare this technique to the classical qualitative diagnosis using two-dimensional electrophoresis (2-DEP) of the glycosaminoglycans (GAGs) in amniotic fluid. Thirty pregnant females each with single fetus were subjected to amniocentesis at 16 weeks gestation. Ten with a previously affected MPS VI infant and twenty served as controls. Prenatal diagnosis (PD) was done by both MS and 2-DEP.

**Results:** MS verified 2-DEP results which showed 5 affected and 5 non-affected fetuses with MPS VI.

**Conclusion:** Two-dimensional electrophoresis of the GAGs in amniotic fluid is a good qualitative method and MS was an accurate quantitative method for prenatal diagnosis of MPS type VI. Quantitative determination of GAGs in AF by mass spectrometry is quicker. Where prenatal diagnosis is recommended for at risk pregnancies, mass spectrometry could be used more in the future as it gives rapid and accurate results.

**Keywords:** Mass spectrometry (MS), Two-dimensional electrophoresis (2-DEP), Prenatal diagnosis of MPS VI, Amniocentesis

## Background

The mucopolysaccharidoses (MPS) are autosomal recessive disorders characterized by deficiency of a lysosomal enzyme which breaks down the glycosaminoglycans (GAGs). This deficiency results in widespread intra- and extra-cellular accumulations of GAGs [1]. MPS disorders

have been subdivided according to the enzyme defect and systemic manifestations [2].

In an Egyptian study done in the National Research Center for the biochemical diagnosis of MPS over 11 years, the most common type of MPS was type VI estimated to be 25.5% [3].

Mucopolysaccharidosis VI (MPS VI) or Maroteaux–Lamy syndrome is an autosomal recessive lysosomal storage disorder [4]. It was originally described in 1963 by Dr. Pierre Maroteaux and Dr. Maurice Lamy and determined by mutations in the arylsulfatase B (*ARSB*)

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gene located in chromosome 5 (5q13-5q14), resulting in reduced or absent activity of arylsulfatase B (ARSB) also called *N*-acetylgalactosamine 4-sulfatase [5]. Clinical manifestations are related to progressive accumulation of dermatan sulfate (DS) [6].

Electrophoresis is used to separate macromolecules based on size and charge. The technique applies a negative charge so proteins move towards a positive charge. Electrophoresis is used extensively in DNA, RNA and protein analysis [7]. Electrophoresis may also be used in diagnosis of mucopolysaccharidosis (MPS) where the accumulation of GAGs provides a natural biomarker for these diseases [8].

Mass spectrometry is done to identify unknown compounds in a given sample. It can also be used for the quantification of known materials [9].

Two-dimensional electrophoresis has been used for long time. It is considered the standard test for the initial diagnosis of MPS. It is a qualitative method. For prenatal diagnosis of MPS, it needs 6–8 ml of amniotic fluid and takes 5 working days. It needs personal experience to do the test and to interpret the results.

Mass spectrometry is recently used as a quantitative method. In prenatal diagnosis of MPS, it needs less amount of amniotic fluid than 2-DEP and takes only 2 working days. It is less person dependent, but it costs more. The use of MS is promising in the future.

### Objective

To introduce quantitative determination of dermatan sulfate using mass spectrometry in the prenatal diagnosis of MPS VI in Egypt and to compare this technique to the classical qualitative diagnosis using two-dimensional electrophoresis (2-DEP) of the GAGs in the amniotic fluid.

### Methods

The study included 30 pregnant females each with single fetus. They were divided into two groups. The first group consisted of 10 pregnant with previous history or family history of one or more affected child with MPS VI seeking prenatal diagnosis. The second group consisted of 20 pregnant seeking prenatal diagnosis by amniocentesis for other indications.

The age of the MPS VI group (ranged from 20 to 42 years with a mean of 32.3 years) is comparable to that of the control group (ranged from 22 to 40 years with a mean of 31.6 years).

Seven patients in the MPS VI group have affected sibling/s, and three patients have an affected family member (one patient has an affected sister and two patients have an affected brother in law). Consanguinity was positive in 100% of MPS VI couples.

Amniocentesis was done in previous pregnancies in 3 out of the 10 MPS VI group by 2-DEP of the extracted GAGs.

Our prospective cohort case control study was performed in the Prenatal Diagnosis and Fetal Medicine Department and the Biochemical Genetics Department, NRC, National Research Centre.

History taking and prenatal counseling were done to each patient at her first visit. A detailed medical history, determination of gestational age and history of viral exposure/illness, bleeding, or use of medication during the pregnancy were obtained. Parental past medical history, consanguinity and 3-generation pedigree analysis were documented. All patients were medically free.

All patients were instructed about the research objectives and methodology. The steps of the work and the details of the procedures were explained to them as well. Then, an informed consent was obtained from each patient.

A detailed ultrasound scan was done to check the viability of the fetus, its development compared to the gestational age, any abnormal features, malformations or anomalies, the site of the placenta and the amount of the amniotic fluid and its index. The ultrasound scans were carried out using General Electric Voluson P8 real-time scan system with a capacity of simultaneous B-mode and M-mode scanning. The carrier frequency was obtained using a broad-band probe which covers a range of frequencies from 5 to 7.5 megahertz (MHz). The real-time obstetric ultrasound examination was performed to the pregnant ladies in the supine position.

Amniocentesis was done at 16 weeks of gestational age under ultrasonographic guidance to withdraw 10 cc of amniotic fluid (using a spinal needle size 22G) for biochemical diagnosis by both 2-DEP of extracted GAGs and mass spectrometry.

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 160 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 [10].

1. Qualitative analysis of glycosaminoglycans (GAGs) using two-dimensional electrophoresis (2-DEP) was done. 0.1–2 µl of the extracted GAGs were applied

on cellulose acetate sheets. The separation proceeded into two phases. The first phase (pyridine, glacial acetic acid, water) for 1 h. The second phase (barium acetate buffer) for 3 h. Staining for 1 h with 0.05% alcian blue solution containing 50 mmol/l  $MgCl_2$  in 50 mmol/l sodium acetate buffer (pH 5.8) and washed with 5% acetic acid solution. Clear blue spots on a white background were obtained [10, 11].

According to the pattern of the detected spots, the diagnosis was made. MPS VI was diagnosed by a big dermatan sulfate spot.

- Quantitative analysis of the GAGs by using Mass spectrometer, (XEVO-TQD, waters, USA).

Dermatan sulfate (DS) stock solution, containing 3 mg/ml, was separately prepared in water and stored at  $-20\text{ }^\circ\text{C}$  until analysis. Calibration curve was prepared, covering a concentration range from 1.56 to 100  $\mu\text{g/ml}$  for DS using seven different levels.

The Ion spray voltage was set to 5500 V, and the temperature was  $300\text{ }^\circ\text{C}$ .

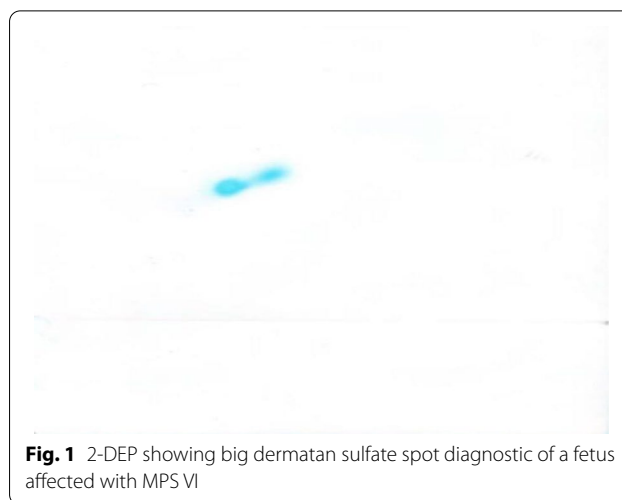
The parent ion of DS is 510.4. The daughter ion of DS is 278.2. The collision energy for DS is 13 V in positive mode.

Chromatographic separation was carried out using a Kinetex Biphenyl column  $2.6\text{ }\mu\text{m}$   $100 \times 2.1\text{ mm}$ , (Phenomenex, Torrance, CA) maintained at  $40\text{ }^\circ\text{C}$  with a flow rate of 0.2 ml/min. Acquisition time was 11 min; the elute was introduced into the ESI interface without splitting. The mobile phase A consisted of 0.1% formic acid in water, while solvent B was 0.1% formic acid in acetonitrile. Total run time was 21 min with gradient elution: 0.0–2.0 min (15% B); 2.1–14.1 min (35% B); 14.2–17.1 min (100%); 17.2–21.0 min (15% B). The injection volume was 1  $\mu\text{l}$  [12].

In MPS VI, affected fetuses dermatan sulfate is elevated.

Pregnants with nonaffected fetuses results in the MPS VI group were seen 1 month after delivery, and the newborns were tested and proved to be nonaffected with MPS VI.

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) Statistical Software. Qualitative data were presented as frequencies and percentages. The Chi-square was used to determine the presence of significant differences between studied groups. *P* values less than 0.05 will be considered as statistically significant. Quantitative data were presented as mean  $\pm$  standard deviation (SD), and student *t*-test was used for statistical analysis.



**Fig. 1** 2-DEP showing big dermatan sulfate spot diagnostic of a fetus affected with MPS VI

**Table 1** Comparison of dermatan sulfate ( $\mu\text{g/ml}$ ) in Amniotic fluid measured by mass spectrometry between nonaffected fetuses, affected fetuses of MPS VI patients and control patients

Compared items	Nonaffected fetuses (5 patients)	Affected fetuses (5 patients)	Control (20 patients)
Min	20.8	663.8	20.7
Max	61.7	705.6	80.9
Mean	47.8	689.5	54.6
S.D	17.7	16.9	18.2
P 1		0.009	–
P 2			0.008
P 3	0.753		

P1: comparison between nonaffected and affected fetuses

P2: comparison between affected fetuses and control

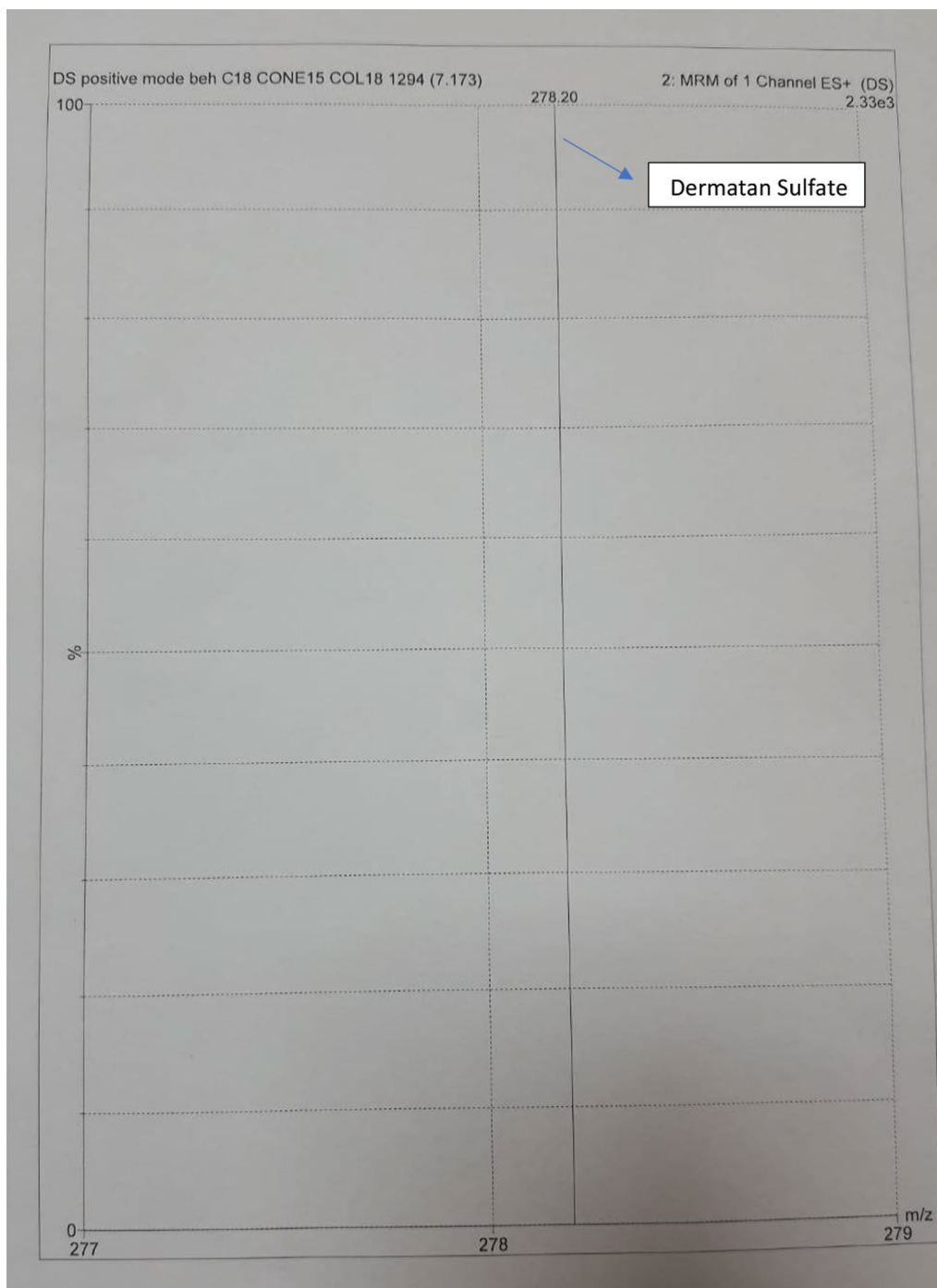
P3: comparison between nonaffected fetuses and control

## Results

2-DEP of the GAGs in amniotic fluid of the MPS VI pregnant showed 5 affected and 5 nonaffected fetuses. Figure 1 shows big dermatan sulfate spot diagnostic of an affected fetus with MPS VI.

Mass spectrometry of amniotic fluid of MPS VI pregnant verified the results of the 2-DEP. Dermatan sulfate was higher among the 5 affected MPS VI fetuses in comparison with the 5 nonaffected fetuses and 20 controls. Differences were statistically significant (Table 1).

Figure 2 illustrates a chromatogram of Dermatan Sulfate by Mass Spectrometry showing daughter fragment release from DS by butanolysis in positive mode.



**Fig. 2** Chromatogram of Dermatan Sulfate by Mass Spectrometry showing daughter fragment release from DS by butanolysis in positive mode

## Discussion

Consanguineous marriages are frequent among Egyptians and lead to high incidence of recessive genetic disorders.

Our results agree with previous Egyptian studies. Positive consanguinity among pregnant with history of MPS was 70% in a study done at the national research center [13] and 78.6% in another Egyptian study [14].

MPS can be diagnosed prenatally by measuring the enzyme activity in chorionic villi or in cultured cells (amniocytes or 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 [15]. 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 [16].

Although two-dimensional electrophoresis has been used for long time as the gold standard for the diagnosis of MPS, it has significant shortcomings such as low load ability and poor separation of hydrophobic, acidic and alkaline proteins [17]. Two-dimensional electrophoresis has been limited by its inability to resolve proteins that are too basic or too acidic, too large or too small, enhanced resolution, detection, quantitation, and reproducibility [18].

The availability of quantitative techniques has facilitated translational research on GAGs into the medical field for diagnosis, monitoring, and screening for MPS. Also, the analysis of GAG synthetic and degradation pathways. The determination of physiological and pathological roles of GAGs is also possible [19].

We have used mass spectrometry for the quantitative measurement of dermatan sulfate in amniotic fluid.

Mass spectrometry showed similar results as 2-DEP concerning the diagnosis of affected and non-affected fetuses in MPS VI.

Table 2 shows the differences between two-dimensional electrophoresis & mass spectrometry used in our study. Both 2-DEP and mass spectrometry techniques are accurate in the prenatal diagnosis of MPS. They need a lot of experience to be implemented and interpreted.

2-DEP of the GAGs in amniotic fluid is a good qualitative method, and mass spectrometry is a new accurate

quantitative method for prenatal diagnosis of MPS type VI.

The amount of AF needed for the extraction of the GAGs and electrophoresis is more than the amount needed for the digestion and quantification by mass spectrometry. Moreover, 2-DEP consumes more time than mass spectrometry. The extraction of GAGs from AF takes 2 working days while the two-dimensional electrophoresis of the extracted GAGs and staining of the plate will take 2 to 3 days more. In total, it takes five working days to get the result.

Quantitative determination of GAGs in AF by mass spectrometry takes less working hours.

Implementing the test and adjustment of the curves needs a lot of experience, but once the curves are adjusted and tuned, results will be available within 48 h.

2-DEP running cost is moderate; while in case of mass spectrometry, the starting and adjustment costs are high beside the high price of the machine itself. Possibly in the future these costs will be less.

## Conclusions

Both 2-DEP and mass spectrometry techniques are accurate in the prenatal diagnosis of MPS. They need a lot of experience to be implemented and interpreted.

2-DEP of the GAGs in amniotic fluid is a good qualitative method, and mass spectrometry is a new accurate quantitative method for prenatal diagnosis of MPS type VI.

Prenatal diagnosis is recommended in all pregnancies with either a previous affected sibling or a positive family history of MPS VI.

Mass spectrometry is recommended to be used for the prenatal diagnosis of MPS VI as it is an accurate, quantitative and rapid method.

Mass spectrometry could be used more in the future for the prenatal diagnosis of all MPS types.

**Table 2** Comparison between 2-DEP & MS

	Two-dimensional electrophoresis (2-DEP)	Mass spectrometry (MS)
1-How long it has been used	Used for long time	Recently used
2-Type of test	Qualitative	Quantitative
3-Amount of amniotic fluid	Needs more	Needs less
4-Duration (working days)	5 days	2 days
5-Experience needed	More personal experience	Training with machine
6-Person-dependency	More	Less
7-Cost	Less	More
8-Prospect	Standard test	Promising use in future

**Abbreviations**

Af: Amniotic fluid; ARSB: Arylsulfatase B; DNA: Deoxyribonucleic acid; DS: Dermatan Sulfate; GAGs: Glucose amino glycans; MPS VI: Mucopolysaccharidosis type VI; MS: Mass spectrometry; PD: Prenatal Diagnosis; RNA: Ribonucleic acid; 2-DEP: Two-dimensional electrophoresis.

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**Authors' contributions**

All Authors contributed to the selection of the study point and study design. Material and data collection was done by AAA author, 2-DEP was done by MMI author, and MS was done by ASG author. The first draft was written by AAA author, while it was revised by other authors. All authors read and approved the final manuscript.

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**Declarations****Ethical approval and consent to participate**

Approval was obtained from the medical research ethical committee at the National Research Centre (The reference number is 18015). The procedures used in this study adhere to the tenets of the Declaration of Helsinki. A signed written informed consent was obtained from all individual participants included in the study.

**Consent for publication**

Not applicable.

**Competing interest**

The authors declare that they have no competing interests.

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