Clinica Chimica Acta 460 (2016) 63–66

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Amniotic fluid glycosaminoglycans in the prenatal diagnosis of mucopolysaccharidoses - A useful biomarker

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ARTICLE INFO

Article history:
Received 30 March 2016
Received in revised form 25 May 2016
Accepted 20 June 2016
Available online 21 June 2016

Keywords:
Amniotic fluid
Glycosaminoglycans
Mucopolysaccharidoses
2-dimensional electrophoresis

ABSTRACT

Background: Amniotic fluid glycosaminoglycan estimation is a useful marker in fetuses affected with mucopolysaccharidoses (MPS). Although known for long, it is not widely used in the prenatal diagnosis. With the availability of more reliable analytical testing and knowledge of normal levels at specific gestations, amniotic fluid glycosaminoglycan at 16–22 weeks of gestation can be a useful biomarker in the prenatal diagnosis of MPS.
Methods: Forty-one women with normal pregnancies were tested for glycosaminoglycan levels in the amniotic fluid and 8 pregnancies with known family history of MPS were tested by sulphated glycosaminoglycan assay.
Results: We established the amniotic fluid glycosaminoglycan levels in normal pregnancies between 16–22 weeks gestation in Indian women. The mean glycosaminoglycan levels were 16.1 ± 8.7 μg/ml. Out of 8 pregnancies with a positive family history of MPS, 2 showed elevated glycosaminoglycans in the amniotic fluid (220 and 410 μg/ml). The lysosomal enzyme assays, i.e., iduronate-2-sulphate sulphatase and β-glucuronidase in these 2 confirmed the diagnosis of MPS II and MPS VII, respectively. In the remaining 6 pregnancies, both glycosaminoglycan levels and enzyme assays were normal.
Conclusions: Glycosaminoglycans are excreted into amniotic fluid by the fetal kidneys and could be used as a marker in the prenatal diagnosis of Mucopolysaccharidoses. This is a useful, fast and cost-effective diagnostic tool in the prenatal diagnosis of mucopolysaccharidoses.

1. Introduction

Lysosomal storage diseases (LSDs) are a group of about 45 different diseases, each characterized by a specific lysosomal enzyme deficiency. Lysosomal enzymes are important in the intracellular degradation of macromolecules to low molecular weight compounds. Deficiencies of these enzymes results in the accumulation of un-degraded macromolecules within the lysosomes causing pathological features of the disease. Mucopolysaccharidoses (MPS) are the most common of all the LSDs. The overall birth prevalence of MPS in the Polish population is estimated to be 1.81 per 100,000 live births, which represents a Central European population, is lower when compared to the prevalence reported for other European countries, such as the Netherlands (4.5 per 100,000 live births) [1]. Prevalence in India is not known but the extrapolated statistical data on MPS I alone in India revealed an incidence of 783 for a population of 1,065,070, 607[2] as compared to 955 for an estimated Chinese population of 1,298,847,624[2]. This is the highest prevalence in India more than the Chinese [2].

The burden of lysosomal storage disorders in India is high as reported in a recent study of LSDs [3]. With the availability of diagnostic facilities, more number of LSDs were being identified across the world and there is an increased demand for prenatal diagnosis (PND). In view of this, we examined the feasibility of developing a cost-effective, reliable and quick method for the PND of MPS based on the concentration of glycosaminoglycan (GAG) levels in cell free amniotic fluid (AMF).

Current PND approach for MPS is expensive and includes lysosomal enzyme assays and mutation analysis. These two approaches are useful in cases when diagnosis is established in the indexed case. In ambiguous cases and where diagnosis is not available, there is a need to have a screening test to rule out MPS. In view of this, we explored the possibility of GAG levels as a prenatal screening marker for MPS disorders. AMF GAGs offer a useful back-up for prenatal diagnosis and should be applied only after 14 weeks of gestation. Earlier, this type of testing was thought to be unreliable for prenatal diagnosis. Now, with the use of more advanced analytical methods and knowledge of specific GAG values at

Abbreviations: LSD, lysosomal storage diseases; MPS, mucopolysaccharidoses; PND, prenatal diagnosis; GAG, glycosaminoglycan; AMF, amniotic fluid; 2-DE, 2-dimensional electrophoresis; CS, chondroitin sulphate; HA, hyaluronic acid; DS, dermatan sulphate; IDS, iduronate-2-sulfatase; KS, keratin sulphate.

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http://dx.doi.org/10.1016/j.cca.2016.06.021
0009-8981/

different gestational periods, this is a useful method as a back-up for prenatal diagnosis. The availability of the test comes handy when the cell culture fail or the culture gets contaminated. In certain parts of the world, neither the enzyme assays nor mutation analysis are available, and estimation of AMF GAGs hold promise in prenatal diagnosis of MPS.

GAGs are long-chain complex carbohydrates that are usually linked to proteins to form proteoglycans which are the major constituents of the ground substance of connective tissue. The major GAGs are chondroitin-4-sulphate (C-4-S), chondroitin-6-sulphate (C-6-S), heparan sulphate (HS), dermatan sulphate (DS), keratan sulphate (KS), and hyaluronic acid (HA). GAGs in AMF are primarily derived from fetal membranes i.e. the amnion and secondarily from the placenta and umbilical cord. Heparan sulphate represents <5% of the total glycosaminoglycans, and dermatan sulphate is generally not detectable, in normal AMF. The pattern of AMF GAGs in affected pregnancies is similar to the post-natal urine of patients with the same disorder except that hyaluronic acid is seen in addition in AMF.

2. Study design

2.1. Materials

2.1.1. Controls

Prenatal samples collected for karyotyping with no family history of MPS were used as normal controls to establish reference ranges for AMF GAGs. Forty-one women with normal pregnancies were tested for GAG levels in the cell free AMF between 16–22 weeks of gestation. A 10 ml of AMF was collected under ultrasound guidance. All these women had no family history of MPS and the sampling was done mainly for karyotyping. The cell free AMF was used to quantitate GAGs. Enzyme assay was performed on the cultured fibroblasts. The study protocol was approved by the Institutional Ethic Committee and written informed consent was obtained from all the parents.

2.1.2. Patients

Eight pregnancies with a confirmed diagnosis of MPS in the proband (MPS I, II, VII, IIIA, VI) were referred for the prenatal diagnosis by enzyme analysis were included in the study.

2.2. Methods

GAG quantification was carried out using "Blyscan Sulphated Glycosaminoglycan Assay" kit (Biocolor Limited, UK), which is a quantitative dye-binding method for the analysis of sulphated proteoglycans and glycosaminoglycan. 2-dimensional electrophoresis (2-DE) was performed with standard Alcian blue reagent method [4]. Enzyme assays were performed by using artificial fluorigenic (4-methylumbelliferyl) and chromogenic substrates [5–14].

3. Results

The study gives reference values for GAGs at 16–22 weeks of gestation. The mean GAG levels for this gestational age was 16.13 μg/ml (Table 1). The 2-DE of normal AMF showed 2 bands, HA and chondroitin sulphate (CS) (Fig 1). Of the eight pregnancies at risk for MPS, GAG analysis and enzyme assays were in agreement. In 2 fetuses,GAGs and enzyme assays confirmed the diagnosis of MPS. On 2-DE, both cases showed DS and HS bands (Fig. 2). In 1 case, GAG level was 220 μg/ml in the AMF and Iduronate-2-sulfatase (IDS) deficiency was observed confirming the diagnosis of MPS II. In the other fetus with nonimmune hydrops, the GAG level was 410 μg/ml and β-glucuronidase was deficient confirming the diagnosis of MPS VII (Table 1). The other 6 pregnancies predicted to be unaffected based on the AMF GAGs and corresponding enzyme assays. The pregnancy outcome was normal.

4. Discussion

Enzyme analysis or mutation studies are the usual methods in the prenatal diagnosis of MPS. This is possible when a definite diagnosis is available in the index case. However, the tests are expensive and not affordable especially in developing countries. Analysis of cell-free AMF offers the advantage of being a rapid, reliable and cost-effective method eliminating the need for culturing the AMF. There is a general agreement that fetal urine is a major contributor in the formation of AMF from mid-pregnancy [15]. Two spots should be present in any 2-DE of normal amniotic fluid. The first is the hyaluronic acid spot indicating its AMF nature and the second is the small chondroitin spot confirming the presence of fetal urine and functioning of fetal kidneys. In MPS I, II and VII, 2 bands representing DS and HS are seen in addition to CS and HA bands. In MPS III and VI, HS and DS bands are visible respectively. In MPS IV, the KS is difficult to visualize and hence the GAG quantification is important. MPS IVA patients also accumulate sulfated hexosamines presumably reflecting the alternative degradative route of KS by β-*N*-acetylhexosaminidase. MPS IV should be considered in the presence of elevated GAGs and a normal 2-DE [16–17].

In view of paucity of normative data on AMF concentration of GAGs among Indian women, we established the normal range of GAG values in the AMF at 16–22 weeks of gestation. At this gestation, enough of fetal urine is excreted and hence useful in assessment. Our study has shown a value of 16.3 ± 8.7 μg/ml in normal pregnancies. Of the 8 pregnancies at risk for MPS, GAG analysis helped in identifying 2 pregnancies, which were later confirmed to be MPS II and MPS VII through specific lysosomal enzyme assays. In the other six cases where GAG levels were in the normal limit, specific enzyme assays were normal and delivered normal neonates. The analysis is sensitive ruling out the possibility of false negative predictions. The test could be positive in other conditions such as Rh isoimmunization, congenital nephrosis,down syndrome and neural tube defects. Hence, GAG elevation suggests either the presence of MPS or the other comorbid

Table 1
Amniotic Fluid GAG levels in normal and suspected MPS pregnancies.

Category	Gestation	No of subjects	GAG	2DE	Enzyme activity (reference range)	Units
Control group	16–22 weeks	41	16.1 ± 8.7	CS, HA	Normal	–
Affected patient group	16–18 weeks	8	–	–	–	–
MPS II	18 weeks	1	228	CS, HS, DS, HA	0.6 (46–263)	nmol/4 h/mg
MPS VII	16–17 weeks	1	410	CS, HS, DS, HA	0.2 (42–175)	nmol/h/mg
Suspected MPS group with positive family history						
MPS IIIA	18 weeks	1	20.8	CS, HA	14.4 (7.3–26)	nmol/17 h/mg
MPS VI	16 weeks	1	23.5	CS, HA	227 (133–459)	nmol/h/mg
MPS I	17 weeks	1	34.4	CS, HA	19 (21–143)	nmol/h/mg
MPS II	18 weeks	1	26	CS, HA	102 (46–263)	nmol/4 h/mg
MPS IVA	16–17 weeks	1	15	CS, HA	10 (7.3–26)	nmol/17 h/mg
MPS IIIA	19 weeks	1	25	CS, HA	16 (7.3–26)	nmol/17 h/mg