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Influence of place of residence on indications for genetic amniocentesis in the Pomeranian region of Poland before and after introduction of the Prenatal Screening Program in 2008

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Background: The aim of the study was to analyze differences in the indications for amniocentesis in patients living in urban and rural areas before and after introduction of the Prenatal Screening Program by Polish National Health Insurance agency in the Pomeranian region in 2008.

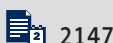
Material/Methods: Indications for 2578 amniocenteses performed in the Department of Obstetrics of the Medical University of Gdansk between 1996 and 2010 were recorded.

Results: Advanced maternal age accounted for 69% of women in urban areas and 61% of women in rural areas being referred for amniocentesis ($p < 0.001$). There was also a significant difference between locations in chromosomal abnormality in previous pregnancy, accounting for 4% of referrals for amniocentesis in urban areas compared with 7% of referrals in rural areas. In urban areas, advanced maternal age accounted for 73% of referrals between 1996-2007 compared with 60% of referrals for amniocentesis between 2008 and 2010 ($p = 0.004$), and in rural areas it was 66% and 54%, respectively ($p < 0.001$). Abnormal result of biochemical screening surprisingly accounted for 13% of referrals for amniocenteses between 1996-2007 in urban areas compared with 28% after 2008 ($p < 0.001$). In rural areas this indication accounted for 12% referrals before 2008 and for 28% from 2008 onward ($p < 0.001$).

Conclusions: The results of the study suggest that in both urban and rural areas there was a significant decrease in advanced maternal age as a reason for referral for amniocentesis, but a significant increase in abnormal results of biochemical screening as an indication for amniocentesis after 2008.

Keywords: amniocentesis • indication • urban • rural, maternal age • biochemical screening • ultrasound screening • fetal malformation • chromosomal abnormality

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Background

Amniocentesis is one of the most popular and safe methods of invasive prenatal diagnosis of chromosomal abnormalities. It is performed in patients with high risk of having a child with birth defects [1–3]. In 2007, the Polish National Health Insurance agency issued new guidelines for prenatal screening. The Pomeranian region was the first to introduce the guidelines in everyday practice in 2008. The main purpose of the Program was described as the early identification of the risk of fetal abnormalities via biochemical screening, the early diagnosis of fetal malformations with ultrasound examination, and the increase of the availability of prenatal screening in Poland. Other aims included the development of a prenatal screening system in Poland, the preparation of an algorithm for non-invasive and invasive prenatal diagnosis, and the improvement of prophylaxis in families with a high risk of genetic disorders through molecular diagnosis and genetic counselling.

To be included in the Prenatal Screening Program, the pregnant woman had to meet at least 1 of criteria listed below:

1. a maternal age of 35 years or more;
2. a chromosomal abnormality in a previous fetus or child;
3. a known structural chromosomal abnormality in the pregnant mother or the father of the baby;
4. a significantly high risk of giving birth to a child with genetic disease conditioned by 1 known gene or with disease with multifactorial conditioning;
5. a fetal malformation found at the ultrasound examination or a high risk of chromosomal abnormality according to the biochemical screening.

Before the introduction of the Prenatal Screening Program, invasive diagnostic procedures were offered to patients with the aforementioned indications. A very low availability of biochemical and ultrasound screening implied that very few patients referred for amniocentesis, chorionic villus sampling, or fetal blood sampling as a result of an abnormal result in the screening. The majority of women undergoing invasive procedures were referred due to maternal age. The National Health Insurance agency hoped that a group of patients over 35 years of age with low risk of fetal chromosomal abnormality in the biochemical screening would not ask for an invasive diagnosis and, on the other hand, that a group of young women with a high risk will undergo amniocentesis. The result of these changes should thus increase the detection of chromosomal abnormalities without increasing the number of invasive procedures [1,2].

The aim of the study was to analyze the differences in the indications for amniocentesis in the patients living in urban and rural areas before and after introduction of the Prenatal Screening Program by the Polish National Health Insurance agency in the Pomeranian region of Poland in 2008.

Material and Methods

Indications for all 2578 consecutive amniocenteses performed in the Department of Obstetrics of the Medical University of Gdansk between 1996 and 2010 were recorded. The Department was the referral center for the Pomeranian region, where the Prenatal Screening Program was first introduced in 2008. All analyzed patients were referred for amniocentesis due to high risk of chromosomal abnormalities.

Every woman referred for amniocentesis was first counselled by a geneticist and signed an informed consent. An ultrasound examination was performed before every procedure. Biparietal diameter, femur length, abdomen circumference, the localization of the fetus and placenta, and amniotic fluid volume were assessed. Amniocentesis was performed using the Yale Spinal 22 gauge needle under ultrasound guidance. The volume of withdrawn amniotic fluid in mL was equal to the gestational age in weeks, as Hanson proposed. In the case of an ineffective first attempt to obtain amniotic fluid, a second attempt was made with the use of a new needle. If the second amniocentesis failed and no amniotic fluid was obtained, the patient was scheduled for a repetition of the procedure 1 week later.

After every amniocentesis, the fetal heart rate was confirmed, and bleeding to the amniotic cavity was excluded. In Rh-negative women with negative Rh antibodies, 300 µg of human anti-RhD immunoglobulin was administered intramuscularly.

In all cases indication for amniocentesis, patient age, and the gestational age were recorded. If there were 2 indications, the one suggesting a greater risk of chromosomal aberration was chosen (for example, if the patient was 36 years old, and biochemical screening gave a risk of 1:20, the biochemical screening was recorded as the first indication for amniocentesis).

In the first part of the study, 2062 (80%) women living in urban areas (group A) were compared to 516 women (20%) living in rural areas (group B).

In the second part of the study, patients were divided into 2 groups. Group I consisted of 1704 women who underwent amniocentesis before the introduction of the Prenatal Screening Program (1996 to 2007). The patients referred from 2008 to 2010 (874 women), after the introduction of the Prenatal Screening Program, constituted group II.

Indications for amniocentesis were compared between group of 1399 urban patients and referred from 1996–2007 (group IA) and 663 women referred after 2007 (group IIA). We also

Table 1. Indications for amniocentesis before and after 2008 in the urban patients.

| Indication for amniocentesis in the urban patients | Group IA 1996–2007 n % | Group IIA 2008–2010 n % | χ^2 test p-value |
|--|------------------------------|-------------------------------|--------------------------|
| Maternal age 35 years or more | 1016 (72.6) | 400 (60.3) | 0.004 |
| Abnormal result of biochemical screening | 178 (12.7) | 183 (27.6) | <0.001 |
| Chromosomal abnormality in previous pregnancy | 73 (5.2) | 18 (2.7) | 0.921 |
| Fetal abnormality diagnosed in ultrasound screening | 37 (2.6) | 39 (5.9) | 0.657 |
| Anxiety and other indications | 47 (3.4) | 11 (1.7) | 0.353 |
| Fetal malformation in previous pregnancy | 33 (2.4) | 4 (0.6) | 0.033 |
| Genetic disease or chromosomal abnormality in family | 15 (1.1) | 8 (1.2) | 0.953 |
| Total | 1399 (100.0) | 663 (100.0) | |

Table 2. Indications for amniocentesis before and after 2008 in the rural patients.

| Indication for amniocentesis in the rural patients | Group IB 1996–2007 n % | Group IIB 2008–2010 n % | χ^2 test p-value |
|--|------------------------------|-------------------------------|--------------------------|
| Maternal age 35 years or more | 201 (65.9) | 113 (53.5) | <0.001 |
| Abnormal result of biochemical screening | 38 (12.5) | 60 (28.4) | <0.001 |
| Chromosomal abnormality in previous pregnancy | 21 (6.9) | 15 (7.1) | 0.009 |
| Fetal abnormality diagnosed in ultrasound screening | 16 (5.2) | 13 (6.2) | <0.001 |
| Anxiety and other indications | 8 (2.6) | 3 (1.4) | NA |
| Fetal malformation in previous pregnancy | 15 (4.9) | 3 (1.4) | NA |
| Genetic disease or chromosomal abnormality in family | 6 (2.0) | 4 (2.0) | NA |
| Total | 305 (100.0) | 211 (100.0) | |

compared 305 rural patients referred before 2008 (group IB) and 211 after 2008 (group IIB) (Tables 1 and 2).

The median age of the patients was 37 years, and ranged from 16 to 50 in group A (urban) and the median age of patients was 36 (ranged from 15–48) in group B (rural). There were 1851 women (71.77%) older than 34 years. Amniocentesis was performed at between 10 and 20 weeks of gestation (average 14 weeks) in group A, and at between 11 and 20 weeks of gestation (average 15 weeks) in Group B, as calculated according to the last menstrual period, Fisher test $p=0.727$.

The data was recorded in the Microsoft Excel 2010 calculation sheet. Statistical software PASW Statistics 18 was used for analysis. Fisher's exact test was used to compare variables when expected differences were small (<5%) and χ^2 test of

independence was used to compare categorical data. The significance level was 0.05.

Results

Table 3 shows that the majority of women were referred for advanced maternal age (35 years or more) – it was the reason of testing in 1417 of the urban women (69%) and in 314 rural patients (61%) ($p<0.001$). The only significance difference between the 2 groups in indication for amniocentesis was chromosomal abnormality of a previous child, which was the indication for amniocentesis for 91 (4.4%) of the urban women compared to 36 (7.0%) of the rural women ($p<0.001$). None of the other indications showed any significant difference between urban and rural women.

Table 3. Indications for amniocentesis in the groups of urban and rural patients.

| Indication for amniocentesis | Group A (urban) | | Group B (rural) | | χ^2 test p-value |
|--|--------------------|---------|--------------------|---------|--------------------------|
| | n | % | n | % | |
| Maternal age 35 years or more | 1417 | (68.7) | 314 | (60.9) | <0.001 |
| Abnormal result of biochemical screening | 361 | (17.5) | 98 | (19.0) | 0.430 |
| Chromosomal abnormality in previous pregnancy | 91 | (4.4) | 36 | (7.0) | <0.001 |
| Fetal abnormality diagnosed in ultrasound screening | 76 | (3.7) | 29 | (5.6) | 0.047 |
| Anxiety and other indications | 57 | (2.8) | 11 | (2.1) | 0.391 |
| Fetal malformation in previous pregnancy | 37 | (1.8) | 18 | (3.5) | 0.350 |
| Genetic disease or chromosomal abnormality in family | 23 | (1.1) | 10 | (1.9) | 0.137 |
| Total | 2062 | (100.0) | 516 | (100.0) | |

Tables 1 and 2 present changes of indications for amniocentesis in urban and rural women after introducing the Prenatal Screening Program in the Pomeranian region in 2008.

Table 1 shows that frequency of performing amniocentesis for advanced maternal age and fetal malformations in a previous pregnancy significantly decreased (from 73% to 60% and from 2.4 to 0.6 respectively, $p=0.004$ and $p=0.033$, respectively). Amniocentesis became more popular due to positive biochemical screening – its frequency increased from 13% to 28% ($p<0.001$). No other significant differences regarding indications for amniocentesis were found between urban and rural women before and after 2008.

Table 2 shows that in the rural women, frequency of referrals for maternal age (66% and 54%, $p<0.001$) significantly decreased. We observed significant increase of referrals for positive results of biochemical screening (12% and 28%, $p<0.001$), fetal abnormality diagnosed in ultrasound (5% and 6%, $p<0.001$), and chromosomal abnormality in a previous pregnancy (6.9% and 7.1%, $p=0.009$).

Discussion

Prenatal cytogenetic diagnosis has been recognized for more than 40 years as a reliable method for the detection of fetal chromosome abnormalities in patients with high risk of having a child with birth defects. Amniocentesis was developed at the end of the 1960s as a diagnostic tool following the ability to culture amniotic fluid samples and, as a result, the first fetal karyotypes were obtained, making this the safest of the invasive procedures [4,5]. The information that the baby does not carry any chromosomal abnormality despite the high risk is essential for the mother. When the abnormality is confirmed,

there is a possibility of termination of pregnancy or time to prepare for having a child with birth defects. Nevertheless, there is a possibility of fetal loss associated with invasive procedures and it should be performed only in patients when the risk of chromosomal abnormality is very high.

Initially, advanced maternal age was the main referral reason for amniocentesis, as it was well known that fetal aneuploidies and maternal age are positively correlated. The combination of maternal age, ultrasound, and biochemical markers used nowadays has changed the paradigm of antenatal screening for Down's syndrome world-wide.

Women choose to have invasive diagnosis for a variety of reasons, mainly for positive screening, but also because of advanced maternal age, a previously affected child, or presence of ultrasound markers of aneuploidy. On average, between 5% and 10% of pregnant women decide to have invasive tests [6,7]. Nowadays, as a consequence of the introduction of effective screening methods, the number of invasive prenatal diagnostic procedures is steadily declining [2,8]. Many women over age 35 or who previously had a child with birth defects choose non-invasive tests and do not insist on invasive procedures if the results were negative. We confirmed this trend in our material – frequency of referrals due to maternal age and affected child in a previous pregnancy decreased after introduction of the Prenatal Screening Program in Poland.

In Poland, advanced maternal age was and still is the most frequent reason for invasive testing. It has been more prominent in urban than rural areas in the analyzed groups. Probably it is not well accepted in rural areas that older women are at higher risk of giving birth to a child with chromosomal abnormality. Additionally, higher frequency of chromosomal abnormality in a previous pregnancy and fetal malformation diagnosed in

ultrasound observed in the group of rural patients suggests that for rural women the indications for amniocentesis have to be stronger than for urban women to decide on invasive testing.

In Poland, all pregnant women should be offered information about screening methods in pregnancy, including a combined assessment of Down's syndrome risk in the first trimester based on maternal age, nuchal translucency measurement, serum free β human chorionic gonadotropin (hCG), and pregnancy-associated plasma protein A (PAPP-A), called the double test or PAPP-A test, by the prenatal care physicians. Until 2007, all patients could have the test, but at their own expense. In 2008 in the Pomeranian region it became free for a group of patients over 35 as a result of introducing the Prenatal Screening Program. This resulted in higher frequency of referrals for invasive testing because of positive biochemical screening, because screening became available for many women who could not otherwise afford it.

Restricting invasive procedures to women at an increased risk has more than halved the proportion of women having an invasive procedure only because of advanced age [9]. We proved that introducing the Prenatal Screening Program in Poland reduced the percentage of women undergoing invasive procedures due to advanced maternal age only. Many of them have a possibility of choosing non-invasive testing and avoid a risk of losing the pregnancy due to amniocentesis when the screening result is negative. More and more women do not see advanced maternal age as a risk factor strong enough to insist on invasive testing.

There are considerable difficulties in planning and investing funds by national health systems where combined screening is concerned. Screening does not lead directly to a reduction

in number of the chromosomal abnormalities, but only helps in the decision as to whether to perform prenatal diagnosis of the chromosomal abnormality, which is neither curable nor preventable. The effect of combined screening is to avoid fetal deaths as a result of invasive diagnostic procedures performed on healthy fetuses, and implementation of this program should be supported at the national level [12]. The results of our analysis of the indication for amniocentesis in urban and rural patients revealed that after the introduction of the Prenatal Screening Program by the National Health Insurance agency in the Pomeranian region of Poland, amniocentesis was performed in both groups more often after an abnormal result of biochemical screening (a significant increase).

Conclusions

Introducing the Prenatal Screening Program in Poland decreased frequency of invasive testing for maternal age or giving birth to a child with malformations in a previous pregnancy. In the rural women, advanced maternal age is less frequently accepted as a reason for high-risk of fetal aneuploidy, which is probably a reason for the lower frequency of patients receiving amniocentesis for maternal age, and higher frequency for fetal malformation in ultrasound screening, genetic disease, or fetal malformation in the family history compared to the urban patients.

High risk detected in biochemical screening is still relatively rarely the reason for invasive testing. It should be emphasized that its popularization in both groups of patients may increase the detection rate. The Prenatal Screening Program needs to be continued for both public health and financial reasons.

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