The results of prenatal screening in the group of 2285 pregnant women from Western Pomeranian Region of Poland diagnosed between 2005-2006

Wyniki przesiewowych badań prenatalnych w materiale 2285 ciąży z rejonu Pomorza Zachodniego diagnozowanych w latach 2005-2006

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Summary

In the following paper we have presented the results of non-invasive and invasive prenatal diagnostic tests performed on 2285 pregnant women from the Western-Pomeranian Region between 2005 and 2006.

Material and methods: Retrospective analysis of screening tests on 2285 pregnant women. Medical history, including age, weight, familial data pedigrees up to third degree relatives, accompanying diseases, gestational complications in the family, type, dosage and period of any drugs intake, was obtained. Sonographic screening and evaluation of maternal serum PAPP-A and $\beta$HCG levels.

Results: Screening tests identified 4.5% high-risk pregnancies in this group. 69% of the patients consented to invasive diagnosis. As a result, genetic anomalies were detected in 43.7% of cases. Significant differences in $\beta$HCG levels correlated with oral gestagens intake and place of residence (coastal areas).

Conclusion: Broad use of certified non-invasive methods of prenatal screening allow substantial reduction of invasive procedures with high levels of positive prediction. Medical drugs intake as well as place of inhabitation may influence on free $\beta$HCG levels.

Key words: prenatal diagnosis – analytic methods / biochemical markers / ultrasound / pregnancy outcome

Streszczenie

Cel pracy: Celem pracy było przeanalizowanie wyników nieinwazyjnych i inwazyjnych badań prenatalnych przeprowadzonych w regionie zachodniopomorskim w latach 2005-2006.

Material i metody: Analiza retrospektywna 2285 ciąży. Wywiad z uwzględnieniem 3 pokoleniowego rodowodu, badania ultrasonograficzne i/wyniast wraz z testem podwodnym. Amniopunkcje ze standardową oceną kariotypu.

Wyniki: Drogą badań przesiewowych wyłoniono 4,5% ciąży podwyższonego ryzyka, z których 69% poddało się amniopunkcji. Pozwoliło to na potwierdzenie w wysokim odsetku (43,7%) nieprawidłowości genetycznych.

W badanej grupie ciężarnych zauważono istotne zmiany w parametrach biochemicznych ($\beta$HCG zależne od przyjmowanych leków (gestageny) oraz od miejsca zamieszkania (okolice nadmorskie).

Wnioski: Powszechne zastosowanie certyfikowanych nieinwazyjnych badań prenatalnych pozwala na ograniczenie liczby przeprowadzanych amniopunkcji przy utrzymaniu wysokiego wskaźnika predykcji. Doustne przyjmowanie niektórych leków oraz miejsce zamieszkania pacjentki może wpływać na poziomy wolnej podjednostki $\beta$HCG.

Słowa kluczowe: diagnostyka prenatalna – analiza / ultrasonografia / markery biochemiczne /
Introduction

Standard prenatal screening protocol comprises NT, NB, DV-flow, levels of maternal serum PAPP-A and free βHCG proteins evaluation [1, 2, 3, 4]. Values of these parameters are influenced by individual features and, possibly, unique characteristics of the patients [5, 6, 7, 8, 9].

In the following paper we have presented the results of prenatal screening tests performed between 2005-2006 in the group of 2285 pregnant women from the Western Pomeranian Region.

Aim of the study

The aim of this study was to analyse the results of prenatal tests performed in our region between 2005 and 2006, as well as to assess their validity. As a value of non-invasive tests, we defined percentage of positive tests, which was confirmed by amniocentesis in low and high a priori risk groups.

Material and Methods

In 2005 and 2006, non-invasive prenatal screening tests were performed on 2285 women from the Western Pomeranian Region in The Unit of Cytogenetics at The Department of Genetics and Pathology, Pomeranian Medical University. The study group consisted of 872 (38.2%) women, at least 35 years of age (with primarily elevated risk, a priori), and 1413 (61.8%) younger pregnant women. Half of these women wished to have the tests done, while others were referred to them by their obstetricians. There was a small, statistically insignificant, number of patients from the region who had been tested in other centres.

Medical history, including age, weight, familial data pedigrees up to third degree relatives, accompanying diseases, gestational complications in the family, type, dosage and period of any drugs intake, was obtained. Retrospective analysis of the place of residence was also performed.

Sonographic screening was performed with Voluson730 PRO by 4 obstetricians – 2 of them certified by Foetal Medicine Foundation (FMF). Simultaneously, maternal serum PAPP-A and βHCG levels were evaluated. Biochemical testing with the use of DPC reagents measured electrochemiluminescence. Medians of the examined parameters were updated concurrently. The final risk assessment was done with the use of PRISCA software. Cut-off risk value was assessed at the level of 1:250.

Patients with elevated risk were given the chance of invasive diagnosis. The presented analysis does not include the results of invasive tests performed due to standard genetic indications.

The results were statistically verified by t-Student test.

Results

The overall number of non-invasive tests amounted to 2285, both in the group of patients at the age of 35+, as well as in the younger group (872 and 1413, respectively). Mean age of the women was 33.3 years.

In the course of 2005 and 2006, there were 32311 labours, including 2694 in the 35+ age group (8.34%) in the entire Western Pomeranian Region.
The results of prenatal screening...

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<th>Table I. Levels of βHCG in studied groups.</th>
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Our analysis covered 7.07% of all pregnancies which resulted in a delivery. Screening covered 32.37% and 4.8% of women with primarily high and low-risk factors respectively.

Complex risk calculation revealed higher risk in 201 (8.8%) women, due to either maternal improper serum biochemical results (in 189 cases – 8.3%) or sonographic features (increased NT in 12 cases – 0.52%). Further sonographic evaluation of the subgroup with abnormal biochemical values reduced their final individual risk in 98 women (52%).

Invasive diagnosis was recommended to 103 (4.5% of all) high-risk pregnant patients (complex risk calculation: age, NT, maternal serum test): 61 cases ≥35y (7% of those ≥35y), and 42 cases <35y (3% of <35y). Amniocentesis was performed on 71 patients (69%): 48 ≥35y and 23 <35y. Chromosomal anomalies were found in 31 (43.7%) of all the examined cases (15 ≥35y and 16 <35y).

In case of 11 pregnant women, abnormal biochemical serum test result was the only high-risk factor indicating the need of invasive diagnosis. Out of these, chromosomal aberrations were confirmed in 9 cases (six trisomies 21, two trisomies 18 and one trisomy 13).

Although the results of the complex test revealed higher risk in case of 32 women, they refused to consent to invasive diagnosis (22 ≥35y and 10 <35y respectively). In this subgroup, further diagnosis included sonographic evaluation in the 20th week of gestation. Markers for the most common trisomies were found in 14 cases. In this subgroup, 9 miscarriages and 2 deaths of the foetus were observed, but were not followed by any further genetic diagnosis. Among the rest, one trisomy 21 and two trisomies 18 have been identified postnatally. The remaining 18 newborns were free of any chromosomal defects and were considered to be false positive results of the complex non-invasive test.

The major factor resulting in final biochemical risk enhancement proved to be high MoM values for βHCG, observed especially in women who, following their obstetricians’ recommendations, had been taking gestagens orally in the early pregnancy. Retrospective analysis showed that 58 patients, suffering from the first trimester bleeding after assisted fertilization or threatening miscarriage, were treated for about 4 weeks with approximately 10 mg of dydrogesterone, orally, twice a day, between 8th and 16th week of gestation. In these patients, mean MoM value was statistically different than in 964 controls and were 2.43 and 1.1 respectively. We also observed statistically significant elevation of free βHCG levels with mean MoM value of 3.83 in 30 patients who live in direct vicinity of the sea (Świnoujście, Międzyzdroje, Mieleno, Dziwnów, Darłowo), when compared to 964 controls without any gestagens treatment, living in other regions. (Table I).

Out of all biochemical screening tests performed, only three were false negative. In one case a child with a simple trisomy 21 was born, the other two were translocation trisomies, and were found by invasive diagnosis – amniocentesis – performed due to improper foetal sonographic features only.

**Discussion**

Non-invasive screening test are performed to distinguish pregnancies with an age-independent higher risk of three most common chromosomal defects. In our centre, we have observed a significantly reduced number of invasive diagnostic procedures in women with age-associated elevated risk.

Among 872 women from the 35+ age group, i.e. with elevated risk a priori, the final high risk was calculated in case of 61 patients (7%) only. Out of them, only 48 decided to have invasive diagnosis, and in 15 cases (31%) chromosomal defects were found. In case of the remaining 811 women, with reduced calculated risk, only 1 had a false negative result and, consequently, a child with the Down syndrome was born.

In 1413 younger women with primarily low-risk, tests revealed 41 pregnancies with higher final risk, which accounts for 2.9% of all primarily low-risk pregnancies. Invasive diagnosis was performed in case of 35 patients and a chromosomal defect was found in 16 cases (45.7%). Among the rest of 1372 women, in 2 cases with negative biochemical test results the amniocentesis was performed solely due to improper foetal sonographic features. The 3 false negative results demonstrate real sensitivity of complex screening tests at the level of 93.9% with revealed of about 90% [1, 2, 10, 11].

In the group of 2285 examined women, 201 (8.8%) with elevated risk were identified. The results of maternal serum biochemical test, particularly high βHCG levels, increase the final risk more often than high NT values. However, in 98 cases with improper biochemical values due to sonographic evaluation and particularly low NT values, primarily high risk was reduced to low, which emphasizes the importance of proper sonographic evaluation [3, 12, 13].

The importance of simultaneous maternal serum biochemical test is obvious, as 11 high-risk pregnancies were identified by thses tests alone, and in 9 of them trisomy was diagnosed. These observations prove once again that relying solely on NT values is not recommended as it is a less reliable and sensitive test [1, 2, 12, 14].

In our centre, where strong emphasis was laid on proper NT level check as well as on FMF audit's, we have reached high level of sensitivity: around 93.9% and 1.8% rate of false positive results. It allows elimination of 52% of potential candidates for invasive techniques in women with primarily elevated biochemical risk and reduction to only 4.5% of all examined women, which corresponds with high effectiveness. Positive prediction rate was around 43.7% and negative prediction rate reached 99.9%. Half of the performed amniocentesis procedures identified a defect in the foetus. Sieroszewski estimates the positive prediction rate at the level of 21.2%. However, in his analysis the major emphasis was placed on the second trimester evaluation tests, which are commonly considered to be less sensitive [15].
Other centres in Poland present even lower values, which may result from the fact that it is easier to reach repeatable values in biochemical tests than sonographic ones [16]. Nevertheless, the tendency to reduce the total amount of amniocentesis is observed worldwide [17, 18, 19].

The influence of many different conditions on both, maternal serum markers levels and MoM corrections in calculation programs has been reported repeatedly. βHCG level depends on weight, ethnicity and diet. Furthermore, smokers have these levels significantly lower than non-smokers [5, 6, 7, 8]. We believe oral intake of gestagens was the major factor elevating the levels of βHCG, contrary to the unaffected levels of PAPP-A. This problem should be investigated in the future in more detail. Levels of βHCG in studied groups compared to controls were significantly higher.

Elevated levels of βHCG in women treated with dydrogestosterone have not yet been described. The mechanism of this influence remains unknown. It is possible that trophoblast cells are not the only ones responsible for the βHCG production, but that mononuclear cells (MNC) of the immune system may secrete significant amounts of this protein. It is well known the βHCG from MNCs stimulates the production of progesterone, which is essential in the early stages of a pregnancy, and possibly the other way round. The mechanisms of this process are described in detail only for the development of the embryo [6, 20, 21, 22].

Numerous research and data analysis were performed on large population and they showed various differences in βHCG levels, which proved to be significantly lower in Asians and Africans, when compared to Caucasians. Therefore, these corrections are taken into consideration in many calculation programmes. Levels of βHCG proved to be slightly lower in the Hispanic population as well. However, no such comparative analysis had been performed among smaller subpopulations [5,23]. Undoubtedly, these corrections are crucial for the assessment of the real risk, since overestimated MoM levels are the major cause of false positive results. According to our results, similarly to large populations, there might also be differences among subpopulations. It is possible that one of the reasons of βHCG growth in small subpopulations are physiological processes of thyroid development, accompanied by high iodine concentrations in coastal areas [23, 24].

About 7.07% of all pregnant women in the region were included in our screening tests programme. Mean frequency of the Down syndrome at the level of 1 in 650 newborns suggests that statistically, among those who were not offered a chance to undergo testing, at least 46 pregnancies with this chromosomal abnormality must have occurred; and about 44 of these, thanks to the sensitivity of the screening, might have been discovered prenatally.

At the end of this paper one should ask, whether it is worthwhile performing non-invasive diagnosis in patients with primarily elevated risk. In some countries the high-risk age was increased to 37 years [2, 25, 26].

Complex Screening Tests, as proposed by Nicolaides, fulfill all criteria of effective identification of the most common anomalies with FPR at 5%. This system is based on the complete elimination of age-related primary risk. Instead, it replaces it with individual calculation of the risk, referring to sonographic and biochemical parameters only [2, 3]. This solution should be applied to all pregnant women.

Economic factors, together with high costs and limitations of invasive diagnosis, play an important role in the choice of appropriate procedures, and stimulate the search for, relatively cheaper, non-invasive diagnosis [25, 26, 27].

We have presented a way of reducing the necessity of invasive diagnosis with low ratio of false negative results. Financial means, otherwise used for unnecessary amniocentesis, might be spent on the popularization of the non-invasive systems, provided that the criteria of high quality standards (highly trained crew, accredited staff and frequent audits which maintain repeatability of the results) are fulfilled.

Therefore, although we have reached satisfactory results of the non-invasive tests, since 2007 we have been using only FMF software and DelfiaXpress – accredited by the FMF system – since neither the method nor DPC reagents have gained accreditation.

Conclusions

1. In our centre, broad employment of non-invasive prenatal screening allowed the reduction of the amniocentesis rate with stable level of positive prediction rate.

2. Oral gestagens intake and place of residence may influence free βHCG levels in the first trimester screening.

References


