Study of biochemical markers during the first trimester of pregnancy among women of Almaty city

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Abstract. One of actual problems is the birth of children with genetic diseases, such as Down syndrome, Edwards syndrome, Patau syndrome, Shereshevsky-Turner syndrome. Study of level of biochemical markers of the first trimester of pregnancy for diagnose of chromosomal abnormalities in fetus was the purpose and objectives of this research. A risk group was identified, including 684 women on the base of results of studies of biochemical markers of the 1-st trimester of pregnancy – PAPP-A and β-hCG. In these pregnant women, PAPP-A and β-hCG concentrations were higher or lower than normal values. It has been shown that decrease in PAPP-A levels and increase or decrease in β-hCG in the 1-st trimester of pregnancy may indicate the presence of a chromosomal abnormality in the fetus. Next, a pattern has been identified that with increasing women age, the risk of having children with chromosomal abnormalities increases. During the study, it was found that PAPP-A levels are reduced at Down and Edwards syndromes and slightly reduced at Shereshevsky-Turner syndrome. Level of β-hCG is significantly higher at Down syndrome, while at Edwards syndrome, on the contrary, it has reduced values; and at Shereshevsky-Turner syndrome the level of β-hCG has a normal value. Changes in levels of these indicators from norm do not always indicate a fetal pathology, but they are grounds for further medical examination.

1 Introduction

Progress has been made in diagnosis and treatment of rare diseases in present stage of development of Republic of Kazakhstan. At the same time there are problems in timely diagnosis of genetic diseases and one of the medical and social problems is the congenital and hereditary diseases. In this regard, Ministry of Health of Republic of Kazakhstan decided to conduct a mandatory biochemical screening of pregnant women to formulate indications of chromosomal pathology of the fetus.

The various factors can influence on development of various congenital diseases caused by quantitative and qualitative chromosome mutations [1, 2, 3]. Development of pathologies can also be influenced by age-related aspects, so a risk of having a child with chromosomal

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abnormalities can gradually increase with increasing of maternal age [3, 4].

Standard 1-st trimester screening takes place at 11-13.6 weeks of pregnancy. Screening consists of a biochemical blood test and ultrasound examination. When conducting screening, the main indicators of the pregnant woman are taken into account – age, weight, height, bad habits, and possible diseases that may affect the results of the examination [4].

The presence of certain deviations in concentration of serum markers in pregnant women at Down syndrome and other some chromosomal abnormalities of fetus is beyond doubt, and may also be a sign of disorder in course of pregnancy [4, 5]. Early information about an unfavorable course of pregnancy can lead to reduction of perinatal mortality in region.

In this regard, the purpose of this study was to determine the level of biochemical markers of 1-st trimester of pregnancy in dried blood spots to identify fetal chromosomal abnormalities among women of Almaty city.

2 Materials and Methods

Biochemical screening was carried out in pregnant women in Human Reproduction Center of Almaty city (http://gcrch.kz/ru), in laboratory of enzyme immunoassay of Medical Genetic department of this Center. All of these analyzes (biochemical screening) are carried out in accordance with the relevant Ethical code. Based on results of the screening, a risk group was identified including 684 women.

The age of pregnant women ranged from 20 to 45 years. Three age groups were formed: group I included women 20-30 years old; group II included women from 31 to 40 years old, and group III included women over 40 years old. Blood tests for biochemical markers were performed between 11 and 13.6 weeks of 1st trimester of pregnancy. The concentration of markers – a free fraction of β-subunit of human chorionic gonadotropin (β-hCG) and plasma pregnancy-associated protein A (PAPP-A) – was determined with using the immunofluorescence analysis in dried blood spots.

To determine the concentration of serum markers, a special computer program “MultiCalc” was used. Special diagnostic reagent kits were used in research work: DELFIA/ AutoDELFIA PAPP-A/ Free hCGb dual DBS kit (Perkin Elmer, Finland). Ultrasound scanning was carried out using an ultrasound diagnostic devices MedisonV-10 and MedisonV-20 (Samsung Electronics Co., Ltd, South Korea).

Statistical processing of the results was carried out using Excel 10.0 analysis package with assessment of the significance of differences according to one-sample Student's t-test. The level of p-value $p \leq 0.05$ was considered as significant. The data in the Tables was presented as mean± SE (standard error).

3 Results and Discussion

Chromosomal diseases mean a large group of congenital human pathologies that differ in clinical characteristics. One of the reasons is chromosomal or genomic mutations. According to modern scientific data 5% of newborns are born with various genetic changes, and 0.5% of which are children with chromosomal diseases. Today about 700 chromosomal aberrations (disorders) have been described, and about 100 of them lead to mental retardation, impaired physical development, as well as to development of various malignant chromosomal diseases [4-6].

Currently, the clinical picture and cytogenetics of chromosomal diseases have been studied quite well, but the mechanisms of pathogenesis are still not fully understood. Also, the pathogenesis patterns of various pathological processes developing in the body as a result of chromosomal disorders have not been clarified.

Various hypotheses have been analyzed: a chromosome nondisjunction; the influence of factors such as race and ethnicity, the age of the mother and father, drug treatment of mothers, bad habits, hormonal and non-hormonal contraception, viral diseases in women and much more. In most cases, we can say that the development of these diseases is genetically determined to one degree or another. It can also be said that maternal age is a biological factor that increases the risk of chromosome nondisjunction, but the mechanisms of this phenomenon are unclear. Analysis of the age of pregnant women and maternal serum markers of the first and second trimester of pregnancy is discussed in both domestic and foreign literature; the results of these studies are contradictory [4, 6, 7].

The main biochemical markers of 1^{-st} trimester of pregnancy are β-hCG and PAPP-A, the quantitative values of which play a large role in the development of fetus [7].

The following results of prenatal screening of pregnant women during 1^{-st} trimester of pregnancy period is presented in Table 1.

Table 1. Total number of examined women and women included in the risk group

Total number of examined women	From them women included in risk group, 684 persons							
	Down syndrome		Edwards syndrome		Shereshevsky-Turner syndrome		Patau syndrome	
	all	%, from total	all	%, from total	all	%, from total	all	%, from total
23978	541	2.28	120	0.2	9	0.1	14	0.2

A total 23978 women were examined, of which 684 women were included in the risk group – who had deviations in biochemical markers from normal level.

For Down syndrome (trisomy 21), the number of women of risk group was 541 persons or 2.28% from the total number of examined women. For Edwards syndrome (trisomy 18) this figure is corresponded to 120 persons or 0.2% from the total; for Shereshevsky-Turner syndrome (X monosomy 45, XO) 9 women were at risk or 0.1% from the total; and for Patau syndrome (trisomy 13) 14 women were at risk or 0.2% respectively from the total.

Women at risk were sent to invasive diagnostics. For socio-economic, epistemological and psychological reasons, 80-90% of any examined women do not undergo this procedure at routine. Among women who were sent to invasive diagnostics, the chromosomal pathology was detected in 31 patients.

During our study, when assessing the risk of pathologies in fetus, in addition to a biochemical blood test, we also analyzed the age of pregnant women. The risk of having a child with a chromosomal disorder caused by aneuploidy gradually increases with maternal age, but increases especially sharply after 35 years [6, 8].

According to the results of our research, it was found that average age of women included in risk group was 34.96±6.98 years old. Age of included women in group I was 20-30 years old - the average age of pregnant women in group I was 26±3.28; group II included women from 31 to 40 years old - average age was 35.5±2.81; group III included women over 40 years old - average age was 42.4±1.17. At analysis of indicators of biochemical screening in different age groups it was revealed that at age of 20-30 years a probable anomaly of fetal development was found in 9 women or amounted to 29.03%. In women over 30 years of age, the presence of probable chromosomal abnormalities in fetus increases, so at age of 31-40 years old the fetal probable anomalies were identified in 12 women (or 38.7%) and over 40 years old of age in 10 women (or 32.3%), respectively.

The same results were found in 2020 study - at the age of 20-25 years of women, an anomaly of fetal development was detected in 5 women, which was 15.6%; at the age of 26-30 years, fetal pathology was detected in 4 women (12.5%). In women over 30 years of age,

the presence of chromosomal abnormalities in fetus increases, so at ages of 31-35 years and over 36 years, fetal anomalies were identified in 8 (25%) and 15 (46.9%) women, respectively. Also many scientists have shown that the development of congenital defects in fetus can be influenced by the age of a mother [7].

Pregnancy-associated plasma protein A (PAPP-A) normally ranges from 0.5 to 2.5 MoM; when level of this indicator decreases the chromosomal abnormalities are diagnosed.

PAPP-A indicators in women at our risk-group ranged from 0.07 MoM to 1 MoM. Our studies showed that in 26 (83.9%) of examined women, PAPP-A was below acceptable values and averaged was on level 0.27±0.12 MoM. In 16.1% (5 women), this indicator was within the normal range and averaged was 0.78±0.17 MoM (Table 2).

Table 2. PAPP-A (MoM) indicators of 1-st trimester of pregnancy in women of different age groups

Indicators	Age groups			p-value
	I age group	II age group	III age group	
PAPP-A indicators are normal	0.77±0.03 (n = 1)	0.77±0.04 (n = 2)	0.78±0.04 (n = 2)	
Low PAPP-A values	0.28±0.01 (n = 8)	0.25±0.01 (n = 10)	0.28±0.02 (n = 8)	≤0.05

Note: n – is the number of patients.

Among women of the I age group, the level of PAPP-A was 0.28±0.01 MoM, among women of the II age group – 0.25±0.01 MoM, and among women over 40 years of age, the concentration of PAPP-A in blood plasma corresponded to 0.28±0.02 MoM. A decrease in this indicator may indicate the development of a number of pathological factors, such as: fading pregnancy; high risk of miscarriage; abnormal development of the neural tube; some types of trisomy, the most famous of which is Down syndrome.

In our studies, 14 examined women were diagnosed with fetal pathology for Down syndrome, and 3 women were diagnosed with Edwards syndrome. In case of intrauterine death of a child and the threat of miscarriage, this test is also informative.

Thus, a decrease in PAPP-A levels in 1^{-st} trimester of pregnancy may indicate an increased risk of developing various problems in fetus, as well as various pregnancy complications [5, 6, 8, 9]. First, low levels of PAPP-A may be associated with an increased risk of chromosomal abnormalities such as Down syndrome (trisomy 21) or Edwards syndrome (trisomy 18). However, additional tests such as ultrasound and genetic testing are needed to further diagnose and confirm such conditions. Secondly, decreased PAPP-A levels may be associated with an increased risk of premature pregnancy loss [9]. Third, since PAPP-A plays important role in development and function of placenta, that a reduced concentration of PAPP-A in blood plasma of women may indicate on possible problems with placental function, which may lead to a lack of oxygen and nutrients for fetus during pregnancy [10, 11].

It is important to note that decreased PAPP-A levels are not a final diagnosis, parameter only indicates a possible increased risk of developing certain problems. Further diagnosis and consultation with a specialist (geneticist, perinatologist, etc.) are necessary to obtain more specific information about possible problems and to develop a pregnancy management plan.

Determining the level of free fraction of human chorionic gonadotropin (hCG) at 11-14 weeks of gestation is also an informative diagnostic indicator [4, 5, 7, 8]. The free β-subunit of human chorionic gonadotropin (β-hCG) is normally in range from 0.5 to 2.5 MoM. The level of this marker in blood plasma also allows to determine the risk of developing certain trisomies: Down syndrome (21 chromosomes), Edwards syndrome (18) and Patau syndrome

(13) [9].

Thus, according to results of our study, hCG value in 31 women was 1.83±0.09 MoM. The lowest investigated hCG level was 0.06 MoM, and the highest level was 8.4 MoM. Of the total number of women at risk, 6 women have elevated hCG levels, which was 19.35%. Average amount of hCG in them was 4.21±0.20 MoM. Also, in 6 (19.35%) of examined women, hCG was below the permissible values and amounted as 0.18±0.009 MoM, and in 19 (61.29%) women, hCG was within the normal range and amounted as 1.64±0.08 MoM.

It is known that development of fetal pathologies can be influenced by age of mother [3, 4], therefore, by analyzing hCG levels in different age groups (Table 3), we found that in group of women 20-30 years old, reduced hCG levels were detected in 2 women and was amounted to 0.32±0.01 MoM. Increased values were also found in 2 women and were equal to 4.07±0.19 MoM. It should be noted that in one woman with low hCG PAPP-A levels were normal, while in other woman they were greatly underestimated. And in patients with elevated hCG levels, PAPP-A levels were slightly reduced. Thus, it can be assumed that increase of hCG and decrease of PAPP-A leads to an abnormality on chromosome 21 (Down syndrome).

In women of age group II, a reduced concentration of hCG was detected in 3 women and corresponded to average as 0.12±0.006 MoM. An increased rate was also found in 3 patients and averaged to 2.92±0.09 MoM. In 2 patients with elevated hCG levels, PAPP-A levels were normal and only one woman had low PAPP-A level, but after next follow invasive diagnostics, in all three women Down syndrome was diagnosed. It was found that in women with lower hCG levels, PAPP-A levels were also reduced. A decrease in biochemical markers in 1^{-st} trimester of pregnancy leads to development of chromosomal abnormalities (Edverts syndrome, etc.).

Table 3. Indicators of hCG (MoM) in 1-st trimester of pregnancy in women of different age groups

Indicators	**Age groups**			**p-value**
	I age group	**II age group**	**III age group**	
hCG readings are normal	1.78± 0.09 (n = 8)	1.49±0.07 (n = 9)	1.51±0.06 (n = 2)	
High hCG values	4.07±0.19 (n = 2)	2.92±0.09 (n = 3)	8.4 (n = 1)	≤0.05
Low hCG values	0.32±0.01 (n = 2)	0.12±0.006 (n = 3)	0.09 (n = 1)	≤0.05

Note: n – is the number of patients.

In one woman aged 41-50 years, the hCG level was reduced and corresponded to 0.09 MoM, and in second woman it was significantly higher than normal and was equal to 8.4 MoM. At the same time, these women showed a decrease in concentration of PAPP-A. Chromosome abnormalities were also diagnosed using these indicators.

So, with low hCG values, there is a risk that the fetus have trisomy 18 (Edwards syndrome), while with elevated values of hCG, there is a risk that fetus have trisomy 21 (Down syndrome). Development of Down syndrome is most characterized by an increase in level of hCG (above acceptable values) and a simultaneous decrease in level of PAPP-A [9, 11, 12]. But it should be noted that according to the results of our studies, both with normal levels of both hCG and PAPP-A, and with an increase or decrease in these indicators, the presence of pathology in the fetus was revealed not only in Down syndrome, but also in other chromosomal abnormalities.

4 Conclusion

The prenatal biochemical screening of pregnant women is carried out at Human Reproduction Center in Almaty city to identify the risk of developing congenital defects and hereditary diseases in fetus. The successful implementation of a screening program for detecting congenital anomalies of fetus had significantly reduced the infant mortality. Today, biochemical screening of pregnant women is carried out on a large scale and it is one of safe research methods that identifies the risks of fetal pathologies in early stages of pregnancy. That is why a screening diagnostics is becoming increasingly important.

During the research work, a screening diagnostics of women in 1-st trimester of pregnancy was carried out to determine the risk of having children with genetic abnormalities and their dependence on age of expectant mother.

In 2023, 23978 women passed prenatal biochemical screening, of which 684 women were included in risk group; and the average age of women was 35 years. Our studies have shown that risk of having children with anomalies gradually increases with maternal age, and the age dependence is most clearly evident for trisomy 21 (Down's disease). For Down syndrome, the number of women of risk group was 2.28% of the total number of examined women. For sex chromosome aneuploidies, the age of parents either does not matter at all or its role is very insignificant.

The following biochemical markers were also studied: pregnancy-associated plasma protein-A and β-hCG in the 1st trimester (11 weeks – 13.6 weeks) of pregnancy, and a comparative characteristic of these markers was carried out.

Deviations in the level of biochemical markers in the 1st trimester of pregnancy – PAPP-A and hCG – can be considered as an unfavorable prognostic sign, leading to dysfunction of fetoplacental complex, development of pathologies in fetus, and formation of pregnancy complications.

Thus, it can be noted that in order to identify the development of chromosomal abnormalities in fetus additional with the results of biochemical screening, it is correct to use the computer programs for calculating of risk of chromosomal disorders, taking into account the individual parameters of each pregnant woman – weight, age, ultrasound of fetus, lifestyle and the presence of diseases.

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