CELL-FREE FETAL DNA IN MATERNAL PLASMA AND NONINVASIVE PRENATAL DIAGNOSIS

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The noninvasive nature of the detection of fetal DNA in the maternal circulation represents the greatest advantage over the conventional methods of prenatal diagnosis. The applications of this methodology involve the detection of the fetal sex, and diagnosis, intra-uterine treatment, and evaluation of the prognosis of many diseases. Fetal cells detected in the maternal circulation have also been shown to be implicated in autoimmune diseases and to represent a potential source of stem cells. On the other hand, with the introduction of a technology that detects the fetal sex as early as at 6-8 weeks of gestation, there is the possibility of early abortion based on sex selection for social purposes. This implies an ethical discussion about the question. The introduction of new noninvasive techniques of prenatal diagnosis and the knowledge of the Nursing Team regarding new methodologies can be of great benefit to the mother and her children, and can help the Genetic Counseling of the families.

DESCRIPTORS: prenatal diagnosis; DNA; blood; stem cells; blood group incompatibility; sex; eclampsia; epigenesis, genetic; ethics

DNA FETAL LIBRE EN EL PLASMA MATERNO Y DIAGNÓSTICO PRENATAL NO INVASIVO

La naturaleza no invasiva de la investigación del DNA fetal en la circulación materna representa una ventaja importante con relación a los métodos convencionales de diagnóstico prenatal. El uso de esta metodología implica la determinación del sexo fetal y el diagnóstico, el tratamiento intra-útero y la evaluación del pronóstico en muchas enfermedades. Las células fetales detectadas en la circulación maternal también pueden ser implicadas en enfermedades autoinmunes y representar una fuente potencial de células madre. Por otra parte, con la introducción de una tecnología que detecte el sexo fetal entre 6-8 semanas de gestación, existe la posibilidad de aborto precoz basada en la selección del sexo para los propósitos sociales. Esto implica una discusión ética previa sobre este problema. La introducción de nuevas técnicas no invasivas de diagnóstico prenatal y el conocimiento del Equipo de Enfermería con respecto a las nuevas metodologías pueden ser muy importantes a la madre y a sus niños, y ayudar al Consejo Genético de las familias.

DESCRIPTORES: diagnóstico prenatal; ADN; sangre; células madre; incompatibilidad de grupos sanguíneos; sexo; eclampsia epigénesis genética; ética

DNA LIVRE FETAL EM PLASMA MATERNO E DIAGNÓSTICO PRÉ-NATAL NÃO INVASIVO

A natureza não invasiva para o feto da pesquisa de DNA fetal em circulação materna representa sua maior vantagem sobre os métodos convencionais de diagnóstico pré-natal. As aplicações desta metodologia envolvem a detecção do sexo fetal e o diagnóstico, tratamento intra-útero e avaliação do prognóstico de várias doenças. Já as células fetais detectadas em circulação materna podem estar envolvidas em doenças auto-imunes e representar uma fonte de células-tronco para as mães. Com a introdução comercial de uma técnica que detecta o sexo fetal entre 6-8 semanas, haveria o risco de abortos precoces devido à seleção do sexo por propósitos sociais, tornando necessária uma discussão prévia sobre os aspectos éticos desta questão. A introdução de novas técnicas não invasivas de diagnóstico pré-natal e o conhecimento das mesmas por parte da Equipe de Enfermagem poderão trazer grandes benefícios para a mãe e seus filhos, bem como auxiliar no Aconselhamento Genético das famílias.

DESCRITORES: diagnóstico pré-natal; DNA; sangue; células-tronco; incompatibilidade de grupos sanguíneos; sexo; eclampsia; epigênese genética; ética

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All pregnant women have the potential risk to present an embryo with anomalies. Therefore, maternal age should be taken into consideration in all pregnancies and fetal morphologic ultrasonography and biochemical tests in maternal blood should always be carried out[1]. However, in special cases, there is the need to use invasive methods, such as chorionic villus sampling (CVS) or amniocentesis, which represents a higher risk for the embryo[2]. For this reason, the technologies involving prenatal diagnosis continue to evolve, with emphasis on research for the development and improvement of noninvasive techniques.

FETAL CELLS IN THE MATERNAL CIRCULATION

The detection of fetal cells in maternal lung parenchyma associated with eclampsia has been described since the 19th century[3], but the presence of these cells in maternal peripheral blood continues to be the subject of intense research. Fetal cells appear early in the maternal circulation during the first trimester and continue to be present throughout gestation. However, the isolation of fetal cells from the maternal circulation is still technically complex. Fetal cells can be present in maternal blood at a ratio of 1:100,000 maternal cells or less (or approximately one per ml of maternal blood in euploid pregnancies)[4]. Another problem, in addition to their small numbers, is the permanence of fetal cells in maternal circulation after the pregnancy. There is a report of the detection of nuclear male DNA in a woman who had her last son 27 years before the study[5]. Other investigations have shown that the persistent cells could have an important role in women's autoimmune diseases. On the other hand, these cells appear to have stem cell characteristics, such as the ability to proliferate and differentiate, which could be of benefit to the mothers[4].

CELL-FREE FETAL DNA IN MATERNAL PLASMA

The quantitative analysis of the free fetal DNA demonstrated that this can compose up to 6.2% of the total DNA present in the maternal plasma[6]. Apoptosis would partially explain the disappearance of fetal DNA from maternal circulation after birth, since this is a fast reaction, concluded within 2 to 3 hours after childbirth. These results (a higher ratio in relation to the maternal material and a faster disappearance from the maternal circulation) demonstrate practical superiority of the search for fetal DNA in maternal plasma in relation to the preparation of fetal cells isolated from maternal whole blood[4, 7].

APPLICATIONS

Many genetic diseases are caused by mutations that result in subtle differences between the sequences of maternal and fetal DNA, such as achondroplasia[8] and â-thalassaemia[9]. Many other clinical applications, especially for single gene disorders, have been described. We will discuss some applications that are important because of their incidence in the population.

Fetal Sexing

Most research groups use sequences of chromosome Y in male embryos as a marker of fetal DNA and standardization of the techniques, due to the fact that a normal woman/mother (46,XX) does not possess this chromosome in her genome. Sexing is also important, mainly for diseases with a recessive X-linked pattern of inheritance, with girls being normal or being carriers of the mutation, but healthy, while boys are normal or affected by the disease. Some authors have used the polymerase chain reaction (PCR) and amplification of Y-specific sequences, mainly highly repetitive sequences or genes like SRY[10]. More recently, the TSPY gene has also been used in studies of small DNA samples[11-12].

Another application of fetal sexing is Congenital Adrenal Hyperplasia (CAH), a disease of genetic origin with an autosomal recessive pattern of inheritance. The most common defect is 21-hydroxylase deficiency. Homozygous girls for the deficiency are born with masculinization of the external genitalia, many times requiring surgical correction. The affected boys present normal external genitalia. Prenatal treatment of CAH with dexamethasone to

prevent genital ambiguity has been successfully used[13]. However, to minimize the side effects, the interruption of therapy has been indicated in the case of affected or normal male embryos and normal female embryos. For this reason, fetal sexing is necessary during pregnancy and is generally carried out by invasive methods. Noninvasive fetal sexing based on free fetal DNA in maternal plasma would bring the additional advantage of early discontinuation of medication in the case of male embryos[10].

Rh alloimmunization

Rh alloimmunization is a very important problem in medical and obstetrical clinical practice, potentially leading to hemolytic disease of the newborn. For pregnant negative Rh patients (15% of the population), a positive Rh embryo involves a 16% risk of sensitization to the Rh antigen. Diagnostic procedures and invasive therapy may be necessary to reduce perinatal mortality of positive Rh embryos[14]. Thus, the early detection of fetal RhD status through fetal DNA in the plasma of negative Rh mothers is of great importance in defining the need for interventions, with known risks of gestational loss, or of gestational immunoprophylaxis.

Pre-eclampsia

Pre-eclampsia is characterized by an increase in arterial pressure and proteinuria after the 20th week of pregnancy. Its potential complications, such as eclampsia, represent one of the most serious problems for maternal and fetal health. According to some authors, there is an increase of fetal DNA in the blood of women with pre-eclampsia in comparison with control groups. The increase in the rates of circulating fetal and maternal DNA would correspond to the degree of severity of the illness and, therefore, the level of fetal DNA may serve as a marker of the prognosis and severity of the clinical picture[7,15]. Although most researchers use the Y-chromosome in this specific application, other non-gender markers have been studied, including epigenetic markers, to improve the number of pregnant women that could be submitted to quantitative investigation[16].

DISCUSSION AND CONCLUSION

Due to the significant risk of invasive prenatal diagnosis, there has been an intensive search for noninvasive techniques of fetal DNA sampling. The detection of fetal cells in the maternal circulation has shown implications in autoimmune diseases and their potential as a source of stem cells for the mothers. However, the methodology available to search for these cells is expensive and complex, due to the permanence of fetal cells in the maternal circulation after pregnancy and their scarcity. In contrast, the isolation of cell-free fetal DNA from maternal plasma is relatively easy and inexpensive and allows for the simultaneous processing of many samples.

The technique of fetal sexing from maternal blood can be useful for the standardization of the methodology and in cases of diseases with a recessive X-linked pattern of inheritance, where only boys would be affected. Intrauterine detection could also lead to an early and optimized treatment of diseases in this group and of other illnesses such as CAH. On the other hand, one problem in introducing a technology that detects fetal sex as early as 6-8 weeks of gestation is the possibility of abortion based on sex selection for social purposes. This may occur due to the possible greater psychological and moral acceptability of an earlier interruption of pregnancy. In Brazil, a couple of services perform fetal sexing commercially, using maternal blood. This implies an ethical discussion about the question.

The early detection of fetal RhD status would be highly useful in gestations of negative Rh patients. Besides the importance of the qualitative methods for many other genetic diseases, the quantification of fetal DNA in the maternal circulation seems to be a potential marker of prognosis in pre-eclampsia.

As described by another group, the pregnant women may be interviewed and the sample blood can be taken in their homes by a nurse[17]. The introduction of new noninvasive techniques of prenatal diagnosis and the nursing team's knowledge about new methodologies for patient orientation can be of great benefit to mothers and children, and can help the genetic counseling of families.

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