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Cell-free DNA as a Clinical Indicator in Maternal Blood

Anne Kanında Klinik Gösterge Olarak Serbest DNA

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Abstract

As many expensive and invasive procedures are used for the diagnosis or follow-up of clinical conditions, the measurement of cell-free DNA is a promising, noninvasive method, which considers using blood, follicular fluid, or seminal fluid. This method is used to determine chromosomal abnormalities, genetic disorders, and indicators of some diseases such as polycystic ovary syndrome, pre-eclampsia, and some malignancies. Cell-free DNA, which are DNA fragments outside the nucleus, originates from an apoptotic process. However, to be used as a marker for the previously mentioned diseases is still under investigation. We discuss some aspects of using cell-free DNA measurements as an indicator or marker for pathological conditions.

Keywords: cfDNA; apoptosis; indicator; maternal blood

Özet

Klinik durumların tanısı veya takibinde birçok pahalı ve invaziv prosedür kullanılır iken, serbest DNA ölçümü; kan, foliküler sıvı veya seminal sıvının kullanıldığı invaziv olmayan ve umut verici bir yöntemdir. Bu yöntem kromozomal anormallikleri, genetik bozuklukları ve polikistik over sendromu, preeklampsi ve bazı maligniteler gibi birtakım hastalıkların göstergelerini belirlemek için kullanılmaktadır. Çekirdeğin dışındaki DNA parçaları olan serbest DNA, apoptotik bir süreçten kaynaklanmaktadır. Bununla birlikte, daha önce sözü edilen hastalıkların bir belirteci olarak kullanılmak üzere hâlen araştırılmaktadır. Bu çalışmada, patolojik durumlar için bir gösterge veya belirteç olarak serbest DNA ölçümlerini kullanmanın bazı yönlerinin tartışılması amaçlanmıştır.

Anahtar kelimeler: cfDNA; apoptoz; gösterge; anne kanı

Introduction

I. The history of cell-free DNA (cfDNA) discovery

Cell-free DNA (cfDNA) was discovered in 1948 by Mandel and Mëtais, who observed the existence of free nucleic acids in plasma (1). They found free DNA and RNA in the plasma of healthy as well as unhealthy people. In 1965, Bendich et al. suggested that cfDNA from cancer tissue could be involved in cancer metastasis. In 1966, Tan et al. found an enormous amount of cfDNA in the blood of patients with systemic lupus erythematosus (2). Technological limitations delayed the confirmation of use of cfDNA as an indicator of diseases. Figure 1 shows the presence of cfDNA in blood (3).

II. Definition of cfDNA

cfDNA means DNA fragments present outside the nucleus of a cell. cfDNA is mainly produced by an apoptotic or necrotic process. The fragments are also present in body fluids, so they can be considered as biological markers of pathological states (4). cfDNA has also been discovered in human seminal fluid (5).

III. Mechanisms of cfDNA Release

Circulating cfDNA is a double-stranded molecule, whose molecular weight is lower than that of genomic DNA. cfDNA can be either short (70-200 base pairs) or long (up to 21-kilo base pairs). Two mechanisms demonstrate the existence of cfDNA in the blood.

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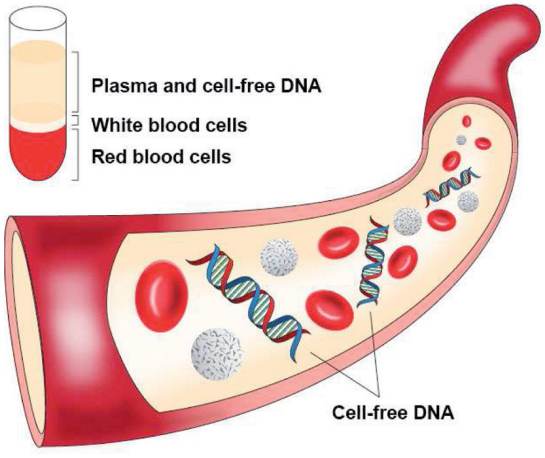

Figure 1: Cell-free DNA in blood (3).

The first mechanism is a passive mechanism because the production of nuclear and mitochondrial DNA is attributed to the damage of apoptotic and necrotic cells (4).
Cell debris is usually phagocytized by macrophages; therefore, cfDNA levels in the blood remain low in healthy persons (6). However, after phagocytosis of necrotic cells, DNA may translocate inside nucleosomes, where it escapes enzymatic degradation (7). This passive mechanism occurs in healthy persons and in people affected by benign diseases.
The other mechanism is an active mechanism that may occur through cellular secretion (8). Figure 2 illustrates the mechanisms of cfDNA release (9).

This review highlights the clinical importance of cfDNA determination.

cfDNA and Maternal Obesity

Obesity is a global problem. Maternal obesity commonly results in pregnancy-related complications such as gestational diabetes and hypertension (10). Evidence has shown that cfDNA level is increased in women with maternal obesity. This increase could be attributed to the increased formation of cfDNA or its decreased clearance in mothers with a high body mass index (BMI). A study reported 1.7% increase in cfDNA level per BMI unit (kg/m^2), and after blood volume adjustment, this increase reached 3.2% (11). In pregnancy, obesity is accompanied by endocrine, metabolic, and immune alterations in adipose tissue. Rapid cellular turnover, commonly seen in obesity, is mainly due to increased death of adipocytes. Therefore, active adipose tissue remodeling causes an increase in maternal cfDNA levels in the circulation of obese mothers (12).

cfDNA in Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy seen in the women of childbearing age. It is characterized by abnormalities of ovarian function and excess androgen production. PCOS is commonly associated with obesity, metabolic disorders, and insulin resistance (13). cfDNA levels are higher in the follicular fluid, collected during in vitro fertilization (IVF), in

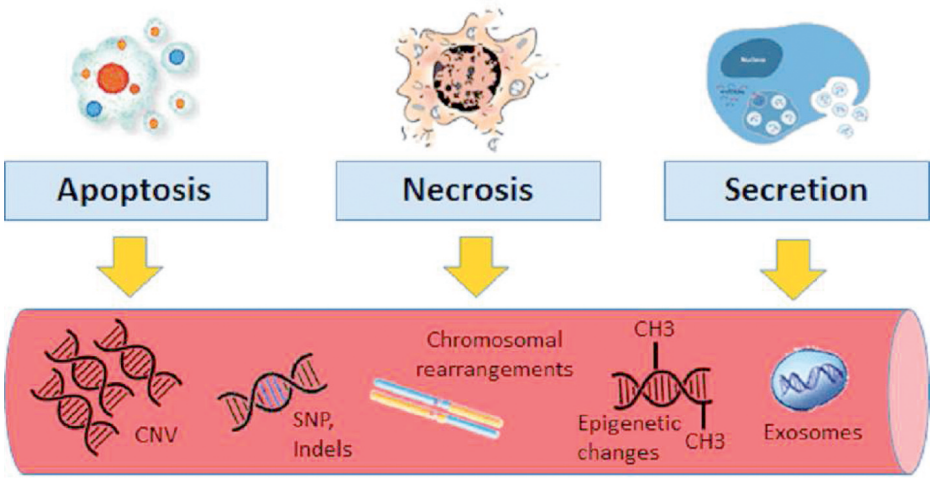

Figure 2: Mechanisms of cfDNA release (9).

women with PCOS than in women with a normal ovarian function. These high levels might be attributed to hyperinsulinemia, which is commonly present in PCOS, increasing apoptosis in granulosa cells (14). In addition, women with PCOS have abnormalities regarding the maturity of follicles as they have a high number of small preantral follicles, which contain higher amounts of cfDNA compared with large follicles (15). Christiansen et al. found that treating pregnant women with PCOS with metformin reduced maternal cfDNA levels (16).

Prenatal cfDNA Screening

This method screens for chromosomal abnormalities in the developing fetus. In the prenatal period, maternal and fetal cfDNA are obtained from the mother's blood sample and examined for the possibility of some chromosome abnormalities, like Down's syndrome, trisomy 13, and trisomy18. This test can also detect fetal sex and rhesus (Rh) type of blood antigen.

Prenatal cfDNA screening is advised for women who are at least ten weeks pregnant. They should be educated about the pros and cons of cfDNA screening (17,18). cfDNA screening has achieved an important role in prenatal care as fetal cfDNA in the blood is used for the early detection of genetic abnormalities, sex of the fetus, and maternal diseases such as pre-eclampsia (19). Figure 3 shows maternal and fetal cfDNA.

cfDNA is considered proinflammatory because of DNA-sensing receptors, like Toll-like receptor 9, and by the stimulation of the interferon pathway. However, the proinflammatory effects of cfDNA on pregnancy and labor have not been proven. An association was found between preterm labor and increased cfDNA levels in the second and third trimesters and at the time of preterm labor (20).

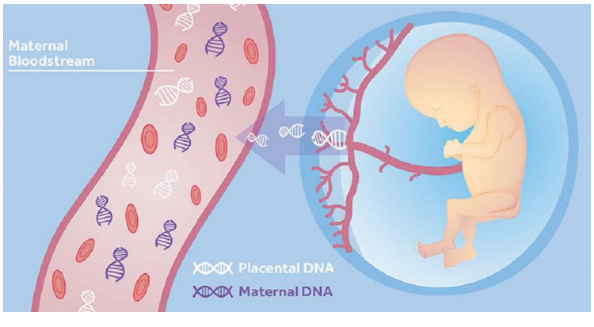

Figure 3: Prenatal cfDNA.
https://embryoplus.gr/en/cell-free-dna-nipt

Applications of cfDNA

1- Prenatal sex determination

The analysis of cfDNA for sex determination is more accurate than obstetric ultrasound and carries a lower risk than amniocentesis. Taking a blood sample from a pregnant woman allows sex determination for the following purposes:

a- Identifying the sex of the fetus permits the decision about the severity of some X-linked recessive genetic disorders, especially if the pregnant woman is a carrier (21).

b- Choosing sex, done by selecting the embryos of the preferred sex after preimplantation genetic diagnosis, or by performing sex-selective abortion according to parents' preference or test results (22).

2- Diagnosis of congenital adrenal hyperplasia

The high secretion of androgens from the adrenal cortex affects fetuses of the female gender (23).

3- Paternity testing

It can be done from six weeks of gestation (24).

4-Diagnosis of single-gene disorders

Some diseases such as cystic fibrosis, beta-thalassemia, sickle cell anemia, spinal muscular atrophy, and myotonic dystrophy, which are autosomal dominant and recessive single-gene disorders, can be detected before birth by the estimation of paternally inherited DNA (25).

5- Diagnosis of hemolytic disease of the fetus and newborn

cfDNA is used to detect fetal RhD in the mother's serum and detection permits the early management of pregnancies at risk of fetal-maternal RhD antigen incompatibility (26).

6- Identifying aneuploidy

Sex chromosomes: Some fetal sex chromosome abnormalities, such as Turner's syndrome, Klinefelter's syndrome, and triple X

syndrome, can be detected by analyzing maternal cfDNA (27).
Other aneuploidies:
-Trisomy 21 (Down's syndrome) (28).
-Trisomy 13 and trisomy 18 (29).

7- Estimating the risk of pre-eclampsia

Pre-eclampsia is a complication that may occur after 20 weeks of pregnancy. It is characterized by hypertension and the presence of protein in the urine. It can be detected early in pregnancy by analyzing cfDNA levels in maternal blood, which increase in the pre-eclampsia state (30).

8- In preimplantation genetic diagnosis

Embryonic cfDNA (a noninvasive test) could be an important source of DNA for the estimation of chromosomal abnormalities (31).

cfDNA in Reproductive Medicine

The main goal of research in reproductive medicine is to discover novel, noninvasive biomarkers that can help improve IVF success rates and lighten causes of IVF failure (32). Serum cfDNA levels of in vitro fertilized women or the culture medium of embryos can be used as a noninvasive indicator of IVF results (33). The level of cfDNA is related to inflammation, which is associated with female infertility (34). A study measured cfDNA levels in the serum of women included in IVF programs, and found that the levels did not alter significantly until the day of a positive pregnancy test (by measuring beta-human chorionic gonadotropin levels in the blood). cfDNA levels were significantly higher in nonpregnant women indicating the possible effect of cfDNA on embryo implantation (35), and this is considered as a beneficial indicator of fertilization success.
Measurement of cfDNA in follicular fluid revealed that cfDNA levels were higher in small follicles (8-12 mm) than in large follicles (>18 mm). Moreover, cfDNA levels in the follicular fluid of top-quality embryos were significantly lower than the levels in follicles of bad quality embryos, suggesting cfDNA as a biomarker of embryos quality (15,36).
A special mechanism increased cfDNA levels in women with infertility who underwent IVF treatment. This induced physicians to search for new interventions to increase the chance of pregnancy in these women (31). High cfDNA levels could be an indicator of high oxidative stress in these women, which has a negative impact on IVF outcomes (37). Stress-related conditions and high cortisol levels in women undergoing IVF programs may contribute to increased cfDNA levels and consequently to IVF failure (38).

cfDNA in Ovaries

cfDNA can be used for the early diagnosis and follow-up of gynecological malignancies; for example, blood cfDNA aids in the diagnosis of epithelial ovarian carcinoma at an early stage (39).
An increased cfDNA level in plasma before the operation is crucial because it may be associated with low survival and it may also be a predictor of mortality from ovarian cancer (40,41). cfDNA, due to promoters, can be methylated, and this methylated cfDNA can also be utilized to distinguish benign from malignant tumors (42). As the tumor increases in size, tumor-specific DNA levels increase and they can decrease after starting chemotherapy (43).

cfDNA and Inflammation

Many studies have demonstrated the association between cfDNA and diseases caused by inflammation (34,43). For example, injury to liver cells caused by an immune reaction might induce the shedding of cfDNA in the circulation in hepatitis B virus infection (44). Furthermore, a recent study reported that acute renal inflammation and injury were associated with high cfDNA levels (45). Many researchers have related the process of apoptosis and plasma cfDNA to inflammation (34,46,47).
cfDNA levels have been associated with the severity of inflammation; chronic inflammations are associated with higher cfDNA levels compared with acute inflammations (48).

Advantages and Disadvantages of Measuring cfDNA

There are some drawbacks in measuring cfDNA levels to use it as a biomarker. One drawback is the low cfDNA levels in the circulation of pregnant women. The other drawback is individual variation in the levels of fetal cfDNA, since the level of maternal cfDNA is more than that of fetal cfDNA (49).

Recent studies have shown that measuring fetal cfDNA carries an increased failure risk because of its low level in maternal blood (50). Some conditions can be missed by fetal cfDNA screening, such as neural tube defects (51).

However, in some conditions such as Down's syndrome, cfDNA has lower false positive rates and higher sensitivity and specificity compared with other traditional screening methods (52). In addition, cfDNA levels can be used more commonly to screen for some chromosomal disorders than usual methods (53). Another advantage is that cfDNA levels can be determined in the pregnancy earlier than other traditional tests (49).

However, the Food and Drug Administration has not yet supported cfDNA measurement and there is not enough evidence concerning cfDNA measurement. Further, the positive results were obtained in commercially funded studies (54).

Conclusion

cfDNA, DNA fragments outside the nucleus, is produced mainly by apoptosis or necrosis. It is present in maternal circulation with relatively higher amounts in obese mothers, women with PCOS, and in some pregnancy complications. cfDNA measurement is a promising noninvasive method of prenatal screening and for the diagnosis of some chromosomal and genetic abnormalities. More recently, cfDNA measurement is also used for the prediction of embryo quality during IVF programs.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Hanan L. Al-Omary, Zainab M. Alawad; Control/Supervision: Hanan L. Al-Omary, Design: Zainab M. Alawad; Data Collection and/or Processing: Hanan L. Al-Omary, Zainab M. Alawad; Analysis and/or Interpretation: Hanan L. Al-Omary, Zainab M. Alawad, Buraq Husseini; Literature Review: Hanan L. Al-Omary, Zainab M. Alawad, Buraq Husseini; Writing the Article: Hanan L. Al-Omary, Zainab M. Alawad, Buraq Husseini; Critical Review: Hanan L. Al-Omary, Zainab M. Alawad; References: Zainab M. Alawad, Hanan L. Al-Omary; Materials: Hanan L. Al-Omary, Zainab M. Alawad.

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