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Cell Free Nucleic Acid: An Approach to Understanding

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Abstract

In developing countries like india, chromosomal anomalies are still the major cause of birth defects accounting for around 3-4% risk. Cell free nucleic acids are currently being studied as a non- invasive method of prenatal diagnosis of monogenic diseases with dominant paternal inheritance, chromosomal disorders, fetal RhD status and sex linked disorders.

Keywords: Cell Free Nucleic Acid; Non Invasive Method; Fetus.

Cell-free nucleic acids are currently being seen as a ray of hope for use as non-invasive biomarkers of gynecological cancers, ovarian, endometrial and obstetric disorders and fetal aneuploidy.

MicroRNAs (miRNAs) are small (19 – 25 nucleotides), single-stranded, non-coding RNA molecules that bind specifically to, and post-transcriptionally regulate, several messenger RNAs (mRNAs) [1].

miRNAs play important physiological roles and miRNA dys- regulation can lead to pathologies. In fertility, miRNAs are associated with the functional regulation of gonadal somatic cells [Leydig and Sertoli cells in testis, and granulosa and cumulus cells (CCs) in the ovary] involved in steroid synthesis.

Cell-free DNA (cfDNA) molecules, which are released mostly by apoptotic or necrotic cells, are also found in body fluids and can be used as biomarkers of pathological conditions [2]. Indeed, cfDNA has been detected in human semen [3].

This cell-free seminal DNA contains DNA epigenetic information that is essential for proper spermatogenesis [4]. Circulating cfDNA in the bloodstream is also being used to detect gynecological abnormalities, whereas fetal cfDNA in maternal blood constitutes a non-invasive biomarker for fetal aneuploidy [5-11].

Role of Circulating miRNAs

• *Circulating miRNAs in Gynecological Disorders and Pregnancy*

Most miRNAs are localized inside the cell; however, a significant number of miRNAs have been detected also in extracellular body fluids, such as serum, plasma, urine, spinal fluid, saliva and follicular fluid [12-16].

These circulating miRNAs could be used as biomarkers of specific conditions, because they are relatively abundant (especially in blood) and quite stable due to their confinement within vesicles where they are protected from RNases.As the amount of specific circulating miRNAs has been associated with tumor development and malignant progression [2], circulating cell-free nucleic acids are now used not only as diagnostic biomarkers, but also as prognostic tools.

Uses of cf- mi RNA in human gynaecological pathologies-

1. Ovarian cancer
2. Breast cancer
3. Endometriosis
4. Polycystic ovarian syndrome
5. Premature ovarian failure[17]

• *Role of miRNAs in the Oocyte – Niche Relationship and in the Hormonal Regulation of Folliculogenesis*

The involvement of miRNAs in the hormonal regulation during folliculogenesis and in the oocyte – niche crosstalk could be exploited for identifying new non-invasive biomarkers of fertility. Moreover, the development of therapies that block the expression or mimic the functions of specific miRNAs may represent a new therapeutic strategy for many gynecological disorders [17].

Role of circulating cell- free DNA

• *Circulating cfDNA for the Non-Invasive Diagnosis of Gynecological and Pregnancy Disorders*

Changes in the levels of circulating DNA have been associated with several diseases, including gynecological and fetal disorders.CfDNA – ovarian cancer, endometrial cancer, maternal obesity, pre-eclampsia/ HELLP syndrome. Fetal cfDNA in maternal circulation – pre-eclampsia/ HELLP syndrome, abnormal placental invasion, preterm delivery, aneuploidy, trisomy [13,18,21], fetal sex determination (x- linked genetic disorders), alpha thalassemia, beta thalassemia, achondroplasia, myotonic dystrophy, cystic fibrosis, huntington's disease, congenital adrenal hyperplasia, hemolytic disease of fetus and newborn, IUGR, hyperemesis gravidarum, polyhydramnios.

Fetal gender determination was the first clinically available test that used analysis of fetal nucleic acids in maternal blood [18]. The main clinical application of fetal gender determination is to aid in the investigation of genetic X-chromosome linked diseases [19,20], such as Duchene's muscular dystrophy and hemophilia, where the confirmation of the female gender excludes the possibility of the fetus carrying such diseases, or even to indicate the treatment of metabolic disorders associated with ambiguous genitalia, such as congenital adrenal hyperplasia (CAH) [21].

Noninvasive genetic fetal RhD genotype testings in RhD-negative mothers through detection of free fetal DNA in maternal plasma was first demonstrated in 1998 by Lo et al [22]. In RhD-negative pregnant women, there is a 16% chance of sensitization to RhD antigen when carrying a RhD-positive fetus, and the consequent development of hemolytic disease of the newborn [23]. As 40% of RhD-negative pregnant women carry fetuses that are also RhD negative, prophylaxis in these cases is unnecessary. Additionally, the product is obtained from human blood (plasma-derived), with a theoretical risk of spreading infection and with the risk of sensitivity reactions [24]. Study in this firld would also be economical, as it would eliminate the costs of unnecessary prophylaxis and the testing for monitoring maternal sensitization, as demonstrated in other countries [25].

The noninvasive PND of monogenic diseases with dominant paternal inheritance is possible due to the absence of the disease-causing allele in the maternal genome, and, so far, has been performed in at least one pregnancy for the following diseases: Huntington's disease [26-28], achondroplasia [29-31], myotonic dystrophy [32], and Apert syndrome [33]. Autosomal recessive diseases are often difficult to diagnose noninvasively, as there is still no way to distinguish between identical maternal and paternal alleles [34]. Several groups have associated an increase in the amount of cffNA in maternal plasma with disorders whose etiology seems to be related to poor placental development. Some examples are preeclampsia [35-37], HELLP syndrome [38], fetal growth restriction [39,40], premature birth [41-42], placenta accreta [43], placenta inccreta [44], hyperemesis gravidarum [45], and polyhydramnios [46].

Role of Nucleic Acids as Emerging Non-Invasive Diagnostic Biomarkers of Female Infertility

• *Circulating miRNAs and Ovarian Function*

Several studies have shown that miRNAs are involved in intercellular signaling [47]. Among the miRNAs expressed in the COC [48], some of them were found also in the plasma [49]. Moreover, the possible relationship between hormonal markers of ovarian reserve/function, at Day 3 of the cycle and circulating miRNA expression could also be assessed. miR-320a was reported to be decreased in follicular fluid from patients with PCOS [16]. High BMI is considered as an indicator of female infertility and the deregulation of some miRNAs has been implicated in obesity [50]. More generally, circulating miRNAs might represent an as yet unexplored tool for the diagnosis/monitoring of infertility/ovarian response.

• *cfDNA as a Biomarker of Ovarian Function*

Based on the finding that the abundance of cfDNA can change in abnormal situations, we hypothesized that variations, particularly an increase, in circulating cfDNA might reflect ovarian reserve disorders.

Recently, it was reported that increased plasma cfDNA levels are asso- ciated with low pregnancy rates in IVF programmes [51].

• *miRNA and cfDNA in Embryo Culture Medium*

miRNAs are involved in the regulation of mammalian embryo development [52,53]. Global miRNA expression profiling suggests that miRNA synthesis and degradation dynamically coexist during preimplantation embryo development [54]. Recently, it has been reported that the presence of cfDNA released into embryo culture medium from mitochondria is associated with poor embryo quality during cleavage [55].

Thus, the study of circulating cell free nucleic acids has opened a new era of research. It is unlocking the importance of non- invasive tools for the management of hazardous diseases of mankind.

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