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Review

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Glycosylated Fibronectin as a wide-spectrum biomarker in pregnancy

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Abstract

Glycosylated Fibronectin (GFN), a glycoprotein involved in cell adhesion and migration, has been implicated in several physiological and pathological processes. Current literature suggests its altered glycosylation patterns might be associated with various pregnancy complications with recent studies providing new empirical evidence from a cohort study involving more than 2,000 pregnant women. The aim of this review, is to summarize the association of GFN levels and its glycosylation patterns with various outcomes including preeclampsia, gestational diabetes mellitus (GDM), spontaneous preterm birth, and intrauterine growth restriction (IUGR) in order to identify potential patterns of the use of GFN as a biomarker for predicting a wide range of pregnancy-related complications, and to provide evidence for further investigation on new diagnostic tools and therapeutic strategies.

Key words: Glycosylated fibronectin, pregnancy complications, biomarkers, protein glycosylation, gestational diabetes mellitus

Introduction

Glycosylated Fibronectin (GlyfN) is a biomarker that has been studied for its potential applications in a variety of pathogenic conditions. At the last decade, it has been widely studied as a biomarker in preeclampsia and gestational diabetes mellitus. A biomarker is defined as a measurable factor that is evaluated in order to examine normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

Fibronectin (Fn) is a glycoprotein present in plasma or serum. It is subdivided into two forms, plasma Fibronectin (pFN) and cellular Fibronectin (cFN). Plasma Fibronectin (pFN), is mainly secreted by the hepatocytes in the liver. Alternatively, cellular Fibronectin (cFN) is formed by fibroblasts, endothelial cells, and smooth muscle cells. Moreover, cellular Fibronectin is detected in the bloodstream during different pathological conditions, such as diabetes

mellitus and inflammation. Given the fact that fibronectin is involved in vessel remodeling and inflammation, specifically glysosylated fibronectin variants potentiality can be used as informative biomarkers.

The disrupted remodeling of spiral arteries in the first trimester leads to inflammation and endothelial dysfunction, which are observed in both gestational diabetes mellitus and preeclampsia. The association between these conditions and glycosylated Fibronectin is due to the shared underlying factors of inflammation and endothelial dysfunction seems to be the mechanism for the increased levels of a specific form of glycosylated fibronectin. Regarding the factors that contribute to gestational diabetes mellitus are typically established early in pregnancy and remain relatively constant. In contrast, the onset and progression of preeclampsia are linked to a gradually rising level of the circumstances that lead to the formation of glycosylated fibronectin.[1]

Molecular mechanism of glycosylated fibronectin

Glycosylated fibronectin is a variant of the fibronectin protein, a large, extracellular matrix glycoprotein is essential for cell adhesion, migration, proliferation, and differentiation. Glycosylation is a post-translational modification in which carbohydrate moieties, such as oligosaccharides, are covalently attached to the protein, altering its structure, stability, and function. The molecular characteristics of glycosylated fibronectin involve the addition of these carbohydrate chains to specific amino acid residues, mainly asparagine (N-linked glycosylation) or serine/threonine (O-linked glycosylation). This glycosylation process can impact fibronectin's ability to interact with other molecules, such as integrins, growth factors, and proteoglycans, thereby modulating cellular processes and influencing tissue remodeling. In the context of pregnancy and associated complications, glycosylated fibronectin has been found to exhibit altered expression patterns, which may contribute to the dysregulation of extracellular matrix dynamics, endothelial dysfunction, and increased inflammation. Further research into the precise molecular characteristics and functional implications of glycosylated fibronectin may lead to a better understanding of its role in pregnancy complications and its potential as a biomarker for conditions such as preeclampsia and eclampsia.

Gestational diabetes mellitus

Gestational diabetes mellitus is a well-known risk factor for the health of both the mother and the fetus and its prevalence is increasing worldwide. According to International Diabetes Federation (IDF), 1 in 6 (16,8%) pregnancies are affected by diabetes. The majority of these cases (86,4%) are complicated by GDM and the remaining 13,6% of these pregnancies are affected by pregestational diabetes. The adverse effects of gestational diabetes mellitus are not limited to the pregnancy but can also have an impact on health both mothers and infants.[2]

The adoption of the new diagnostic criteria based on the recent HAPO study is expected to increase the prevalence of gestational diabetes mellitus to approximately 18% of all pregnancies. About 5-10% of women with gestational diabetes mellitus are diagnosed with type 2 diabetes immediately after pregnancy, while the presence of obstetrical history of gestational diabetes mellitus is associated with a 10-fold higher chance of developing diabetes within the next 10-20 years. Moreover, the children of mothers with gestational diabetes mellitus have an 8-fold higher risk of developing type-2 diabetes mellitus in later life.[3]

Thus, several studies have demonstrated that glycosylated fibronectin can be measured in serum collected in the first trimester and used for the prediction of gestational diabetes mellitus with high accuracy.

Nagalla et al. proposed utilizing fibronectin-SNA as an indicator of GDM during the first trimester of pregnancy. They discovered that the GDM group (n=15) had considerably higher levels of glycosylated fibronectin (fibronectin-SNA) than the control group (n=14). A noteworthy result is that the mother BMI prior to pregnancy did not significantly differ between the two groups. A single marker test with fibronectin-SNA (AUROC: 0.81) seems to be a cost-effective approach in preventing GDM and reducing the increased economic cost associated with its complications.[3]

Camille et al. studied the potential use of glycosylated fibronectin for the screening of gestational diabetes mellitus. Specifically, the study included 182 pregnancies and the researchers measured the serum levels of glycosylated fibronectin which was mainly collected during the first trimester. The authors demonstrated that, with a cut-point of 107 mg/L, the assessment of glycosylated fibronectin is characterized by a sensitivity of 81% and a specificity of 90%. Compared to the 50g glucose loading test, glycosylated fibronectin can be used as an alternative option for the screening of gestational diabetes mellitus.[4]

According to Rasanen et al. found a variation of glycosylated fibronectin levels between the GDM group (90 participants, 132±36 mg/L) and the control group (92 participants, 80±4.0 mg/L, p<0.001), which suggests that glycosylated fibronectin levels could be a useful early predictor of gestational diabetes mellitus. This correlation remained unchanged despite various factors such as maternal age, parity, gestational age at sample collection, and other biomarkers such as adiponectin, high-sensitivity CRP, and placental lactogen. Glycosylated fibronectin's specificity and sensitivity performances suggest that it could be a potentially useful screening test for GDM in the first trimester, with a performance that may be better than that of the 50-g, 1-hour OGTT. Glycosylated fibronectin also showed a significant independent association with GDM (P,.001).

Other biomarkers like adiponectin, high-sensitivity CRP, and placental lactogen performed poorly in classification, compared to glycosylated fibronectin (with respective AUCs of 0.63, 0.68, and 0.67 in comparison to AUC of 0.91 for glycosylated fibronectin). The glycosylated fibronectin levels above a threshold of 120 mg/L accurately identified 57 GDM cases, with a positive predictive value of 63% (95% CI 53–72%) and a negative predictive value of 95% (95% CI 94–95%) at a population prevalence of 12%.[5]

Eclampsia

The early diagnosis of pregnancies with preeclampsia is crucial. The most common neurologic complication is eclampsia which is characterized by convulsions or unexplained coma. The prevalence of eclampsia is reported to be 0.3%. Maternal complications include cerebral hemorrhage, pulmonary edema, placenta abruption, hemorrhage, hepatic rupture, acute kidney injury and cardiovascular disorders. Neonatal complications include neonatal mortality and growth neurodevelopment impairment, neutropenia, leucopenia, thrombocytopenia, increased red blood cells and reticulocytes. While glycosylated fibronectin has been investigated as a potential biomarker for various pregnancy-related complications, there is limited direct evidence for its use in eclampsia prediction specifically. The majority of studies focus on its association with preeclampsia, which is a precursor to eclampsia in some cases. These studies show that glycosylated fibronectin may be a promising marker for identifying pregnant women at increased risk of developing preeclampsia. As the current body of research on glycosylated fibronectin and eclampsia prediction is limited, further investigation is needed to establish its role in this context. Combining glycosylated fibronectin with other known biomarkers and clinical factors may

offer a more robust predictive model for eclampsia and other pregnancy complications.

Preeclampsia

Preeclampsia is a major hypertensive disorder of pregnancy that is associated with severe maternal and perinatal complications. Moreover, pre-eclamptic women are at increased risk for subsequent cardiovascular disease. Specifically, pre-eclampsia complicates 5% of all pregnancies worldwide. Pre-eclampsia and pregnancy-related hypertension diseases are the second most frequent causes of maternal mortality, accounting for over 30.000 maternal deaths annually. Also, pre-eclampsia accounts for 25% of stillbirths and 25% of neonatal deaths.[7,8] Alterations of maternal serum glycosylated fibronectin (GlyFn) levels have been studied in preeclamptic patients (Table 1).

Huhn et al demonstrated that the serum levels of glycosylated fibronectin appear to be elevated during all trimesters of pregnancy in preeclamptic patients. Specifically, the levels of GlyFn were reported to be predictive of preeclampsia with an AUC of 0,94 in the ROC analysis. As a result, this serum test was further recommended as a point-of-care biomarker to quickly determine the risk for preeclampsia. Specifically,151 women with risk factors or clinical signs and symptoms of preeclampsia were included in this study. Maternal serum samples were collected between 20 and 37 weeks of gestation. The clinical diagnosis of preeclapsia was confirmed 21% of women within 4 weeks. GlyFn exhibited a good classification performance (area under the curve (AUROC) = 0.94, 91% sensitivity, 86% specificity).[9]

In a study of Wang et al, GlyFn levels were also found to be higher in the group complicated by preeclampsia. However, this difference was not significant and the authors concluded that this statistical insignificance may be attributed to the sample size, patient recruiting criteria or testing methodology compared to previous studies.[7]

Rasanen et al correlated the levels of GlyFn in maternal serum with the disease severity and the likelihood of poor maternal and fetal outcomes, including higher blood pressure, earlier delivery and lower birthweights. This study aimed to assess the potential use of glycosylated fibronectin (GlyFn) as a biomarker for the prediction and diagnosis of preeclampsia. The study enrolled 107 pregnant women, including 45 normotensive women and 62 with preeclampsia.

The study found that in normotensive women, there was no significant change in GlyFn levels over the course of pregnancy. In contrast, women with preeclampsia had a significant increase in GlyFn levels, with a greater increase observed in those with severe preeclampsia. The study also found that GlyFn levels were associated with various adverse pregnancy outcomes, including preterm delivery, low birth weight, and higher blood pressure.

Furthermore, the study demonstrated that GlyFn levels were a sensitive and specific biomarker for the diagnosis of preeclampsia. A threshold of 176.4 mg/mL was identified, with a positive predictive value of 47% and a negative predictive value of 89%. Additionally, the study found a significant association between high GlyFn levels and small-for-gestational age infants in women with preeclampsia.

However, the study did not find a significant association between high GlyFn levels and the development of HELLP syndrome or placental insufficiency, although a notable percentage of women with high GlyFn levels did develop these complications.

Overall, these findings suggest that GlyFn levels may have clinical utility in predicting and diagnosing preeclampsia, as well as identifying women at increased risk for adverse pregnancy outcomes. However, further research is needed to validate these findings and determine the optimal threshold for clinical use.

According to a recent study, glycosylated fibronectin can also be found in umbilical cord blood. DiPrisco et

Table 1. Glycosylated Fibronectin as a biomarker.

AUTHOR	NUMBER OF PATIENT(N)	TYPE OF MARKER	OUTCOMES
Rasanen J et al (2014)	107	Preeclampsia	The study found that GlyFn serum levels in women with preeclampsia during 1st trimester were significantly higher , and these higher levels persisted. Additionally, there was a significant association between increased levels of glycosylated Fibronectin and several adverse pregnancy outcomes, including gestational age at delivery, blood pressure, and small for gestational age neonates. The weekly change in GlyFn levels between 33-38 weeks was 81.7 mg/mL for participants with mild preeclampsia and 195.2 mg/mL for participants with severe preeclampsia.
Alanen J et al (2020)	79	Gestational diabetes mellitus	there was no statistically significant difference in maternal serum GlyFn levels between women with gestational diabetes mellitus and control women. The median serum GlyFn levels in women with GDM was 447.5 µg/mL (interquartile range 254.4-540.9 µg/mL) and in control women was 437.6 µg/mL (interquartile range 357.1-569.1 µg/mL).
Nagalla SR et al (2015)	1463	Gestational diabetes mellitus	The levels of fibronectin-SNA were significantly elevated in the gestational diabetes mellitus group (P = 0.006).
Rasanen JP et al (2013)	182	Gestational diabetes mellitus	increased glycosylated Fibronectin concentrations in the 1st trimester of pregnancy have been shown to be significantly linked with a higher chance of getting gestational diabetes.
Wang J et al (2021)	196	Preeclampsia	GlyFn was increased, but not significantly (P=0,061), in the patient group that developed preeclampsia.
Nagalla SR et al (2020)	798	Preeclampsia	levels of GlyFn were significantly associated with preeclampsia (PE) ($p<0.01$).
Huhn EA et al (2016)	151	preeclampsia	An AUROC of 0.94 suggests that GlyFn is a strong predictor of preeclampsia, and the sensitivity and specificity values of 91% and 86%, respectively, suggest that it performs well in identifying true positive cases while avoiding false positives.
Di Prisco et al (2020)	196	GlyFn in umbilical blood - preeclampsia	The average levels of GlyFn in the umbilical cord blood were significantly lower in infants born to mothers with preeclampsia compared to those without the condition (119.12 ± 5.9 vs. 155.5 ± 3.9 µg/mL, $p<0.001$). Even after adjusting for maternal and neonatal variables, associations remained substantial.

al, demonstrated that the cord blood levels of glycosylated fibronectin are found to be decreased in neonates born by preeclamptic mothers, in opposition to the increased maternal serum levels. The authors focus on analyzing GlyFn in the umbilical cord blood on the fetal side of the placenta, researchers can gain a better understanding of the pathophysiology of preeclampsia and gain knowledge about the relationships between the condition and its effects on the fetus. The authors carried out research on of 196 neonates, with 49 consecutively enrolled infants born by mothers with preeclampsia. In comparison to controls, neonates born to moms with preeclampsia had considerably lower GlyFn levels (119.12 ± 5.9 vs. 155.5 ± 3.9 μg/mL, $p<0.001$). Remarkably, the association between Glycosylated Fibronectin (GlyFn) and preeclampsia was observed regardless of whether the mother had gestational diabetes. Specifically, in the 70% of patients there was no correlation between neonatal blood glucose levels and GlyFn measurements.[10]

Conclusions

Preeclampsia has been the subject of extensive research, although only a few serum prediction markers have been successfully used in clinical practice. Glycosylated fibronectin seems to be a robust biomarker for monitoring of preeclampsia and gestational diabetes mellitus, and potentially a wide-spectrum biomarker for more pathogenic processes. However, larger well-designed studies in prospective settings are required to further evaluate the utility of glycosylated fibronectin as a screening method for pregnancy disorders both alone and in combination with other markers to evaluate its potential as a diagnostic or prognostic tool.

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