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ZONULIN LEVELS IN COMPLICATED PREGNANCY : A SYSTEMATIC REVIEW

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Abstract

Hepatocytes, enterocytes, adipose tissue, and immune cells are the primary sources of zonulin secretion. It is the major protein responsible for the regulation of tight junctions between intestinal epithelial cells. Stimulating the release of zonulin dissociates zonula occludens-1 protein from tight junctions, resulting in increased intestinal permeability. Intestinal barrier failure has a role in gestational diabetes and intrahepatic cholestasis of pregnancy pathogenesis (ICP). Dietary decisions have a significant impact on intestinal permeability and, as a result, the risk of developing metabolic diseases. The inability of this experiment to account for dietary components and make the appropriate adjustments to the results is a drawback. Throughout the course of our experiment, we noticed that plasma zonulin levels and body mass index (BMI) were positively associated. On the other hand, the conclusions of the studies conducted on pregnant women are debatable. Individuals with problems of pregnancy, such as gestational diabetes, have elevated zonulin levels. As we all know, gestational diabetes can lead to macrosomia, birth canal trauma, and a future risk of diabetes for both mother and child.

Keyword: *Body Mass Index; Compicated Pregnancy; Gestational Diabetes; Metabolic; Zonulin*

INTRODUCTION

At least four intercellular junctions are responsible for regulating the paracellular intestinal permeability, which is responsible for controlling the gaps between epithelial cells. Tight junctions are the junctions that have been studied the most and characterized in the most detail; these junctions are regulated by more than 50 proteins.[1] Increased zonulin levels are thought to be a marker of compromised intestinal barrier, and zonulin is the only detectable blood protein that represents the intestinal permeability. Eukaryotic cells produce zonulin, which acts similarly to the zonula occludens toxin produced by Vibrio cholerae.[2,3]

Similar to prehaptoglobin-2, human zonulin (a protein with a molecular weight of 47 kDa) interacts to protease-activated receptor 2 (PAR2) and epidermal growth factor receptor (EGFR) in the intestinal epithelium.[4] Tight junction disassembly in the small intestine is set into motion by this complex, which triggers the phosphorylation of zonula occludens proteins. The liver is the primary organ responsible for the secretion of zonulin, but other organs and tissues (including enterocytes, adipose tissue, the brain, heart, immune cells, the lungs, the kidney, and the skin) also contribute.[5,6]

Since zonulin raises intestinal permeability, it facilitates the flushing out of bacterial colonization in the gut, which is why gluten and bacteria cause it to be secreted in the first place.[7] Zonulin is primarily released from hepatocytes, enterocytes, adipose tissue, and immune cells. It is the primary protein regulating tight junctions between intestinal epithelial cells. Stimulation of zonulin release dissociates zonula occludens-1 protein from tight junctions, resulting in enhanced intestinal permeability. Intestinal barrier dysfunction has a role in the pathophysiology of gestational diabetes and intrahepatic cholestasis of pregnancy (ICP).[2,8,9]

The purpose of this study endeavor is to determine the levels of zonulin that are present in pregnancies that are difficult.

METHODS

Protocol

The author made sure that this research met the standards by using the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 guidelines. This is done to make sure that the investigation's findings are correct.

Criteria for Eligibility

This literature review examines zonulin levels in complicated pregnancy. This is accomplished by assessing or studying previous research on the topic. The purpose of this essay is to demonstrate the significance of the discussed difficulties. Researchers who participated in research satisfied the following criteria: 1) In order for the manuscript to be accepted for publication, it must be written in English and focus on zonulin levels in complicated pregnancy. 2) This review contains articles published after 2017 but prior to the time frame this systematic review examines. Research that is not permitted includes editorials, submissions without a DOI, already published review articles, and entries that are almost identical to previously published journal papers.

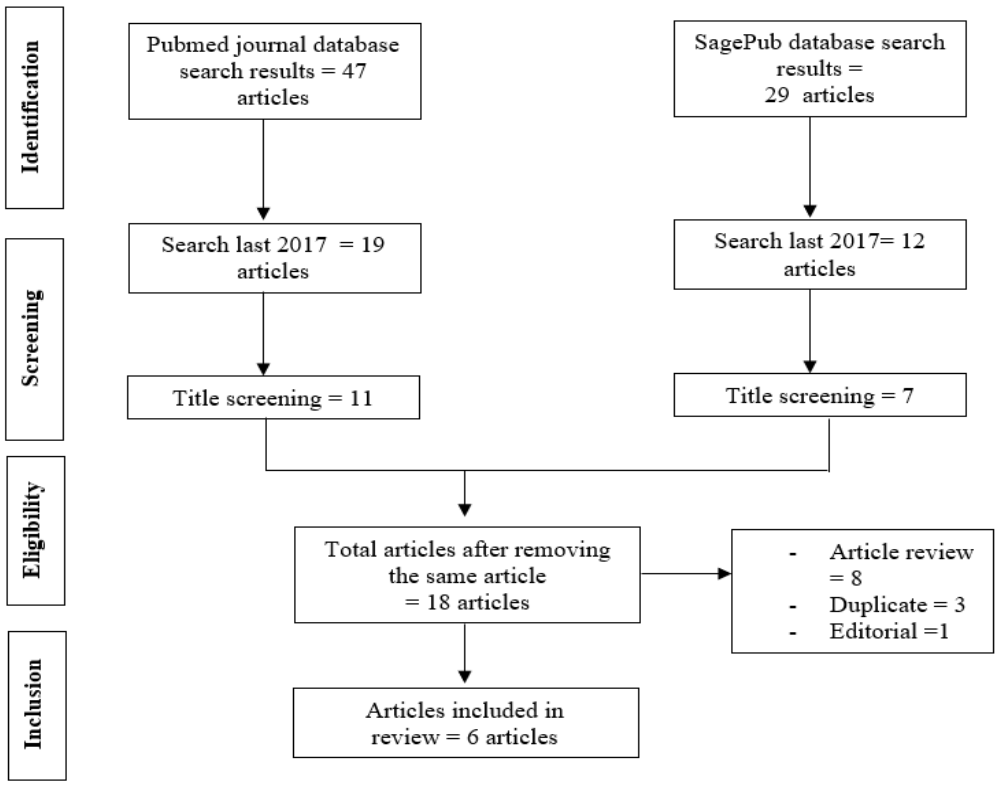

Figure 1. Article search flowchart

Search Strategy

We used "zonulin level" and "complicated pregnancy" as keywords.The search for studies to be included in the systematic review was carried out from February, 23rd 2023 using the PubMed and SagePub databases by inputting the words: *("zonulin"[Supplementary Concept] OR "zonulin"[All Fields]) AND ("level"[All Fields] OR "levels"[All Fields]) AND ("pregnancy complications"[MeSH Terms] OR ("pregnancy"[All Fields] AND "complications"[All Fields]) OR "pregnancy complications"[All Fields] OR ("complicated"[All Fields] AND "pregnancy"[All Fields]) OR "complicated pregnancy"[All Fields])* used in searching the literature.

Data retrieval

After reading the abstract and title of each study, the writers determined if it matched the inclusion criteria. The authors then selected historical works to utilize as sources for this topic. After evaluating multiple studies that all exhibited the same pattern, this conclusion was reached. All submissions must be written in English and have never been published before.

Only studies that met all inclusion criteria were examined in the systematic review. This refines the search results to only include relevant results. Research findings that do not match our criteria are not considered. Following this, the research will be studied in depth. Throughout the investigation for this study, the following information was discovered: names, authors, publication dates, location, study activities, and parameters.

Quality Assessment and Data Synthesis

Each author conducted their own study on the studies given in the publication's title and abstract before picking which publications to investigate further. Then, we'll examine all papers that satisfy the review's inclusion criteria and are, therefore, worthy of inclusion. Then, we will select which publications to include in the review depending on our findings. This criterion is used to determine which manuscripts will be evaluated. To simplify the selection of papers for review as much as possible. What prior studies were undertaken, and what elements of those investigations made them eligible for inclusion in the review?

RESULT

According to Guvey, et al (2021)[10] findings, the plasma zonulin levels of patients diagnosed with ICP (group 1), GDM (group 2), and GDM combined with ICP (group 3) were significantly greater than those of patients diagnosed with healthy pregnancy (group 4, $p < 0.001$) The patient group with the highest median plasma zonulin levels was group 3 (110.33 µg/mL). This was found across all patient groups. The severity of ICP and unfavorable pregnancy outcomes were both linked with zonalin levels. There was a correlation between high blood zonulin levels and gestational diabetes, intracranial hypertension, and unfavorable perinatal outcomes. The presence of both GDM and ICP was responsible for an increase in the serum zonulin levels.

Mokkala, et al (2017)[11] conducted a study with 88 patient. They showed serum zonulin was related with increased odds of gestational diabetes (adjusted odds ratio [aOR] for 1 µg/ml rise in zonulin= 1.08, 95% confidence interval [CI] = 1.02–1.15; P=0.009), as determined by a 2-h 75-g oral glucose tolerance test in late pregnancy. With a sensitivity of 88% (95% CI = 71–100%) and specificity of 47% (95% CI = 33–58%), the best cutoff value was 43.3 µg/ml. The area under the ROC curve was 0.67 (95% CI = 0.54–0.80). They results indicate a correlation between early-pregnancy zonulin levels and GDM, suggesting that zonulin may be a predictor of GDM.

Table 1. The litelature include in this study

Author	Origin	Method	Sample Size	Result
Guvey, 2021[10]	Turkey	Prospective case-control study	Group 1: 95 pregnant women (ICP); group 2: 110 pregnant women (GDM); group 3: 16 women (GDM+ICP); and group 4: 136 healthy pregnant women	The severity of ICP and unfavorable pregnancy outcomes were both linked with zonalin levels. There was a correlation between high blood zonulin levels and gestational diabetes, intracranial hypertension, and unfavorable perinatal outcomes. The presence of both GDM and ICP was responsible for an increase in the serum zonulin levels.
Mokkala, 2017[11]	Finland	Cohort prospective	88 pregnant women	They research reveals a correlation between an elevated early-pregnancy serum zonulin concentration and GDM; hence, our findings point to zonulin as a candidate for the role of a GDM predictor.
Bawah, 2019[9]	Ghana	Prospective longitudinal study	314 pregnant women	Zonulin levels are shown to be significantly elevated throughout the first trimester of pregnancy in women who have GDM, and these elevations occur before the start of GDM.
Mokkala, 2017[12]	Finland	Cross-sectional study	100 pregnant women	The results indicate that increased serum zonulin levels, i.e., greater intestinal permeability, leads to metabolic endotoxemia, systemic inflammation, and insulin resistance in obese pregnant women.
Demir, 2019[13]	Turkey	Prospective observational study	Eighty-five consecutive pregnant subjects	The results indicate that zonulin may be a noninvasive biomarker involved in the pathophysiology of GDM.
Deniz, 2021[14]	Turkey	Prospective case-control study	88 pregnant women (44 patients with ICP and 44 controls)	According to the findings of this study, greater levels of zonulin are linked to unfavorable perinatal outcomes, a more severe form of ICP, and a lack of response to treatment in patients with ICP.

Bawah, et al (2019)[9] showed a study that zonulin levels were high in women with GDM, with a sensitivity of 80.95%, specificity of 80.41%, and a cutoff point of > 47.5 µg/mL for predicting GDM. This study found that zonulin is a good predictor of GDM, with a positive predictive value (PPV) of 0.708 and a negative predictive value (NPV) of 0.986. After taking into account the mother's age and BMI, it was found that obese pregnant women with high plasma zonulin were 109 times more likely to get GDM than those with normal BMIs.

Mokkala, et al (2017)[12] showed that serum zonulin concentration was positively correlated with LPS (P = 0.02), inflammatory markers (P <0.001), insulin (P <0.001), insulin resistance (P <0.001), and triglycerides (P = 0.001), and negatively correlated with insulin sensitivity (P = 0.001) (ANOVA, P <0.015). In the univariate linear regression, both LPS and GlycA demonstrated a positive connection with insulin resistance, serum insulin, triglycerides, total and LDL-cholesterol and a negative relationship with insulin sensitivity (P <0.03). There was also a correlation between LPS and HDL-cholesterol (P = 0.03).

A study in Turkey showed the plasma zonulin level was found the group with GDM to be statistically substantially greater than in the control group (p <0.001). Plasma zonulin level was found to have a positive correlation with body mass index (BMI), creatinine, fasting plasma glucose, baseline, first hour, and two hours glucose levels and the OGTT, hemoglobin A1C (HbA1C), homeostatic model assessment for insulin resistance (HOMA-IR), and alanine aminotransferase (ALT) levels, according to an analysis of correlations.[13]

Women with ICP had significantly higher zonulin levels than controls (mean 0.728 ± 0.520 µg/mL vs. 1.303 ± 0.63 µg/mL, p <0.001). The area under the curve (AUC) for the predictive value of zonulin levels for ICP, as determined by the receiver operating characteristic (ROC) study, was 0.761% (95% CI = 0.661%–0.881%. Serum zonulin levels were independently associated with adverse perinatal outcomes (OR = 1.278, 95% CI = 0.232–7.041), severity ICP (OR = 7.535, 95% CI = 1.597–13.553), and also unresponsiveness to treatment in ICP (OR = 4.178, 95% CI = 0.971–7.818), as determined by multivariable logistic regression analysis.[14]

DISCUSSION

Zonulin has been suggested as a potential novel circulating measure for intestinal permeability, and studies have shown that women who have type 2 diabetes have higher quantities of the protein than healthy women do. The primary finding of this research was that plasma zonulin levels tended to be elevated during pregnancy with higher blood glucose.[3,5] This finding was found to have a positive correlation with BMI, creatinine, fasting plasma glucose, first hour, and two hour glucose levels in the OGTT, in addition to HbA1C, HOMA-IR, and ALT levels.[13]

Investigations have been conducted on a substantial number of subjects in relation to the link that exists between GDM and zonulin. In a study that was prospective and involved 88 pregnant women, it was discovered that the level of zonulin in the serum was connected with GDM. The cut-off value of 43.3 µg/mL possesses a sensitivity of 88% while only having a specificity of 47%. The researchers that carried out this investigation came to the conclusion that zonulin is a valid indicator of GDM.[10,11]

Conversely, a prospective study with a larger sample size examined the plasma zonulin levels of 314 pregnant women at 24–28 gestational weeks using 50 g of OGTT. The plasma zonulin levels of pregnant women with GDM were substantially greater than those of healthy subjects. The zonulin cutoff value was 47.5 ng/mL, with a sensitivity of 80.95 percent and a specificity of 80.41 percent.[9] Among women who are going to develop gestational diabetes, there is an obvious spike in zonulin levels during the first trimester of pregnancy, and this spike occurs before the onset of GDM.

The blood zonulin concentration was found to have a positive correlation with inflammatory markers, insulin, and insulin resistance in a study that was carried out by Mokkala et al.[12] on a group of one hundred obese pregnant women in the form of a cross-sectional investigation. Plasma zonulin levels of patients diagnosed with GDM were considerably greater than those of the control group, according to the findings of a prospective observational study that included a total of 90 pregnant women who did not have GDM and 85 pregnant women who were diagnosed with GDM. Both the research that was discussed earlier and the inquiry that was done produced outcomes that were consistent with each other.[13]

The ratio of lactulose to mannitol in the urine is a clinical diagnostic of gut permeability that is extensively used. Circulating zonulin has a strong correlation with this ratio, and as a result, it is regarded to be a non-invasive biomarker for gut permeability.[5] The zonulin that is seen in circulation originates from a variety of different tissues. However, in order to evaluate the etiology of the elevated zonulin levels and the source of the increased levels, it is necessary to ascertain the degree to which enhanced intestinal permeability occurs during pregnancy.[15]

Ohlsson, et al (2017)[16] revealed that there is no association at all between the amounts of zonulin in the serum and in the feces. As secretion of zonulin from the intestinal barrier may leak into the lumen, this suggests that feces zonulin may have a stronger association with the permeability of the intestinal tract. An compromised intestinal barrier is thought to be reflected by increased zonulin levels as a marker for the condition. It is possible that the intestine is the site of origin for the putative mechanism by which zonulin could contribute to the beginning of gestational diabetes.

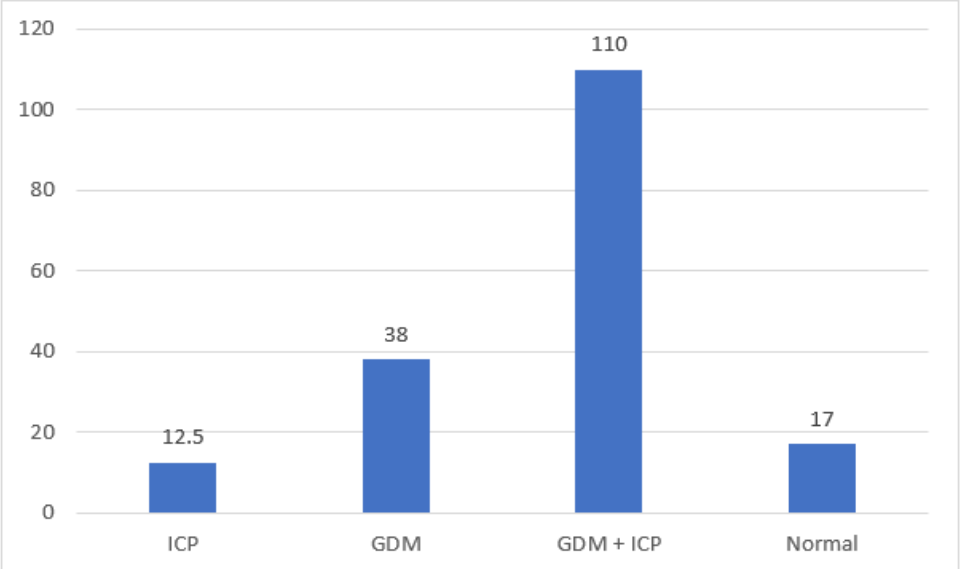

Figure 2. Comparison of zonulin levels in patients with ICP, GDM, and Normal

The intestinal permeability and, as a consequence, the risk for metabolic illnesses are both affected to a great degree by the choices that are made regarding diet. The fact that this study did not account for dietary components and do the necessary adjustments to the results is a limitation of the investigation. Throughout the course of our investigation, we discovered that the levels of plasma zonulin and the BMI were positively correlated with one another. The findings of the research conducted on pregnant women, on the other hand, are up for debate.[17]

Houttu et al,[17] there was no statistically significant difference in the levels of serum zonulin, which is a measure of intestinal permeability, between overweight and obese pregnant women. Intestinal permeability, which was measured by serum zonulin concentration, was found to have a correlation with metabolic endotoxemia, indicators of inflammation, and the metabolism of glucose and lipids in pregnant women who were overweight. Zonulin may have a significant role in intestinal permeability, in the induction of undesirable metabolic reactions, and as a result, may have the potential to affect the health of both the mother and the child.[17]

It is likely that it will be possible to modify maternal metabolism while the mother is pregnant, with subsequent health benefits, if the intestinal barrier is strengthened. A larger waist circumference, greater diastolic blood pressure, higher fasting glucose, and an increased risk of metabolic disorders are all connected with higher zonulin levels, according to research. The richness and composition of the gut microbiota, as well as the intake of n–3 PUFAs, fiber, and a variety of vitamins and minerals, are related with the serum zonulin concentration in obese pregnant women.[18]

Altering the make-up of the microbiota in the gut and the food could have a positive impact on intestinal permeability and, as a result, may have an effect on the health of mothers and their children. There are significant changes in intestinal permeability that occur during pregnancy, along with an increase in zonulin levels. These changes may play a significant role in the observed increases in gestational inflammation, and as a result, may be a potential contributor to the development of gestational diabetes.[18]

Increasing insulin resistance is part of the physiological changes of pregnancy. This is believed to occur to provide the supply of nutrients needed for the growth and development of the fetus. This increase in physiological insulin resistance gradually increases during the second half of pregnancy and decreases rapidly after birth. The increased resistance of gravids to insulin is thought to arise from a combination of increased maternal adiposity and the effects of placental hormonal products.[19]

This progressive increase in insulin resistance is offset by increased levels of insulin secreted by pancreatic β-cells. Islet cells have an extraordinary ability to adopt changes in insulin resistance by increasing insulin production, so that glucose homeostasis is maintained during normal pregnancy and glucose levels are affected only slightly by changes in strong insulin resistance. However, glucose homeostasis is a continuum of action.[20]

There is a gradual decline between normal blood sugar levels, although the physiological state of insulin resistance changes leading to gestational diabetes and ultimately to diabetes mellitus in later life. The obese population suffers from elevated blood glucose levels, insulin resistance, and high rates of diabetes. Obese women were characterized by peak postprandial glucose levels, increased 1 and 2 hour postprandial glucose levels, increased time intervals for peak glucose which were significantly higher, while mean blood glucose at night was significantly lower.[20,21]

Maternal hyperglycemia during pregnancy is considered to be one of the most important predictive factors of pregnancy complications in the obese population. It is now recognized that other maternal parameters associated with obesity and/or overnutrition during pregnancy also include hyperglycemia and hypertriglyceridemia. Changes in the endocrine environment associated with obesity (increased levels of insulin, androgens and leptin) are also associated with a number of disorders of maternal metabolism, such as insulin resistance, diabetes and increased blood pressure.[20]

Macrosomia is defined as birth weight >90 percentile for gestational age or >4000 grams. Macrosomia occurs in 15-45% of infants born to diabetic women, a 3-fold increase in normoglycemic control. Birth weight is largely determined by maternal factors apart from hyperglycemia, with the most significant influences being gestational age at delivery, body mass index (BMI) before pregnancy, maternal height, gestational weight, hypertension, and smoking.[22]

Trauma injuries to newborns associated with macrosomia are shoulder dystocia, clavicular fractures, brachial plexus injuries, reduced Apgar scores for five minutes, long labor intervals, and the need for emergency care for macrosomic infants. Shoulder dystocia that occurs in macrosomic infants is a complication of childbirth, which affects about 10-15% of vaginal deliveries of babies weighing more than 4500 grams at birth.[22]

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CONCLUSION

Patients with pregnancy complications such as GDM and ICP have higher than normal zonulin levels. Gestational diabetes, as we know, can cause macrosomia and is a cause of birth canal trauma and the risk of DM in the future for both mother and child.

REFERENCE

[1]. Chiba H, Osanai M, Murata M, Kojima T, Sawada N. Transmembrane proteins of tight junctions. Biochim Biophys Acta (BBA)-Biomembranes. 2008;1778(3):588–600.
[2]. Drago S, El Asmar R, Di Pierro M, Grazia Clemente M, Tripathi A, Sapone A, et al. Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand J Gastroenterol. 2006 Apr;41(4):408–19.
[3]. Fasano A, Not T, Wang W, Uzzau S, Berti I, Tommasini A, et al. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. Lancet [Internet]. 2000;355(9214):1518–9. Available from: https://www.sciencedirect.com/science/article/pii/S0140673600021693
[4]. Tripathi A, Lammers KM, Goldblum S, Shea-Donohue T, Netzel-Arnett S, Buzza MS, et al. Identification of human zonulin, a physiological modulator of tight junctions, as prehaptoglobin-2. Proc Natl Acad Sci. 2009;106(39):16799–804.
[5]. Sapone A, De Magistris L, Pietzak M, Clemente MG, Tripathi A, Cucca F, et al. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. Diabetes. 2006;55(5):1443–9.
[6]. Fasano A. Regulation of intercellular tight junctions by zonula occludens toxin and its eukaryotic analogue zonulin. Ann N Y Acad Sci. 2000;915:214–22.
[7]. El Asmar R, Panigrahi P, Bamford P, Berti I, Not T, Coppa G V, et al. Host-dependent zonulin secretion causes the impairment of the small intestine barrier function after bacterial exposure. Gastroenterology. 2002;123(5):1607–15.
[8]. Fasano A. Zonulin, regulation of tight junctions, and autoimmune diseases. Ann N Y Acad Sci. 2012;1258(1):25–33.
[9]. Bawah AT, Seini MM, Yakubu YA, Ussher FA, Amoah BY, Alidu H. First trimester zonulin levels and adiposity as predictive indices of gestational diabetes mellitus. Int J Diabetes Dev Ctries. 2019;39:451–7.
[10]. Güvey H, Çelik S, Çalışkan CS, Yılmaz Z, Yılmaz M, Erten Ö, et al. How Do Serum Zonulin Levels Change in Gestational Diabetes Mellitus, Pregnancy Cholestasis, and the Coexistence of Both Diseases? Int J Environ Res Public Health. 2021 Nov;18(23).
[11]. Mokkala K, Tertti K, Rönnemaa T, Vahlberg T, Laitinen K. Evaluation of serum zonulin for use as an early predictor for gestational diabetes. Nutr Diabetes. 2017;7(3):e253–e253.
[12]. Mokkala K, Pellonperä O, Röytiö H, Pussinen P, Rönnemaa T, Laitinen K. Increased intestinal permeability, measured by serum zonulin, is associated with metabolic risk markers in overweight pregnant women. Metabolism. 2017;69:43–50.
[13]. Demir E, Ozkan H, Seckin KD, Sahtiyancı B, Demir B, Tabak O, et al. Plasma zonulin levels as a non-invasive biomarker of intestinal permeability in women with gestational diabetes mellitus. Biomolecules. 2019;9(1):24.
[14]. Deniz CD, Ozler S, Sayın FK. Association of adverse outcomes of intrahepatic cholestasis of pregnancy with zonulin levels. J Obstet Gynaecol J Inst Obstet Gynaecol. 2021 Aug;41(6):904–9.
[15]. Wang W, Uzzau S, Goldblum SE, Fasano A. Human zonulin, a potential modulator of intestinal tight junctions. J Cell Sci. 2000;113(24):4435–40.
[16]. Ohlsson B, Roth B, Larsson E, Höglund P. Calprotectin in serum and zonulin in serum and feces are elevated after introduction of a diet with lower carbohydrate content and higher fiber, fat and protein contents. Biomed reports.

2017 Apr;6(4):411–22.
[17]. Houttu N, Mokkala K, Laitinen K. Overweight and obesity status in pregnant women are related to intestinal microbiota and serum metabolic and inflammatory profiles. Clin Nutr. 2018;37(6):1955–66.
[18]. Mokkala K, Röytiö H, Munukka E, Pietilä S, Ekblad U, Rönnemaa T, et al. Gut microbiota richness and composition and dietary intake of overweight pregnant women are related to serum zonulin concentration, a marker for intestinal permeability. J Nutr. 2016;146(9):1694–700.
[19]. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2016;62–9.
[20]. Salzer L, Tenenbaum-Gavish K, Hod M. Metabolic disorder of pregnancy (understanding pathophysiology of diabetes and preeclampsia). Best Pract Res Clin Obstet Gynaecol [Internet]. 2015;29(3):328–38. Available from: https://www.sciencedirect.com/science/article/pii/S1521693414002119
[21]. Tomsett KI, Barrett HL, Dekker EE, Callaway LK, McIntyre DH, Dekker Nitert M. Dietary Fiber Intake Alters Gut Microbiota Composition but Does Not Improve Gut Wall Barrier Function in Women with Future Hypertensive Disorders of Pregnancy. Nutrients. 2020 Dec;12(12).
[22]. Cunningham FG, Leveno KJ, Bloom SL, Cunningham FG; Leveno KJ; Bloom SL; et al, Cunningham FG, Leveno KJ, et al. Williams Obstetri. 25th ed. New York: The McGraw-Hill Companies; 2020.