GLUT-9 expression in human term placentas of diet regulated gestational diabetes mellitus pregnancies

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GLUT-9 EXPRESSION IN HUMAN TERM PLACENTAS OF DIET REGULATED GESTATIONAL DIABETES MELLITUS PREGNANCIES

DIPLOMA THESIS

Academic year:
2021/2022

Mentor:
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Split, July 2022

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ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my mentor Assist. Prof. Sandra Zekić Tomas, MD, PhD for guiding and helping me write this diploma thesis, as well as being a great teacher and person.

A special thanks to my parents whose endless love and support made me come this far and without whom this would not be possible. To Andrej for always believing *in me and of skroz to my Split Family who made this journey the best of my life.*

To the University that thought me so much and finally to Split for being the best second home I could ever wished for.

1. INTRODUCTION

1.1 Diabetes Mellitus

Diabetes mellitus is a heterogenous metabolic disorder which leads to chronic hyperglycemia caused by defects in insulin secretion, action or both, and thereby results in disturbances of carbohydrate, fat and protein metabolism (1). According to CDC (Centers for Disease Control and Prevention) approximately 415 million people around the world live with diabetes (2). WHO (World Health Organization) approximates it to be even higher, 422 million worldwide, with a higher incidence in low- and middle-income countries (3). In year 1980 there was 108 million people with diagnosed diabetes in the world and the number rose to 422 million people in the world by the year of 2014. An increase of almost 4 times in 34 years that can be largely explained by changes in lifestyle in addition to better screening programs. In the year 2019, 1,5 million deaths were directly caused by diabetes (4).

There are several pathophysiologic processes that can lead to diabetes. The vast majority of diabetes cases can be divided into the two most common types, type 1 in which there is an absolute deficiency of insulin secretion caused by an autoimmune destruction of β-cells of the pancreas and type 2 in which there is a combination of insulin resistance and relative insulin deficiency. There are however many other categories of diabetes which include genetic defects of β-cell function, genetic defects of insulin action, endocrinopathies, gestational diabtes ect (5). It has now been proven that both type 1 and 2 can occur in all age groups and is no longer divided into childhood type 1 and adult type 2, as thought before. Both types shares a common feature of hyperglycemia and the chronic complications are thereby the same although speed of progress may vary (6). Diagnostic criteria for diabetes mellitus is shared by both types and includes: a random plasma glucose of >11.1 mmol/L with symptoms, a fasting plasma glucose >7.0 mmol/L, a HbA1c value >6.5% or abnormal glucose tolerance test, and abnormal values need to be present at least at two different occasions (7).

1.1.2. Type 1 diabetes mellitus

Previously known as ”juvenile-onset” or ”insulin dependent” diabetes mellitus, is caused by cell-mediated autoimmune destruction of β-cells of the pancreas. It accounts for 5-10% of all diabetic patients (5). Age of first detection of number, specificity and titer of autoantibodies are all factors that determine the rate in the which the disease progresses (6). The persistent presence of at least two islet autoantibodies proven by studies of first- degree relatives of patients with type 1 diabetes involve GAD (GAD65), and autoantibodies to the

tyrosine phosphatases IA-2 and IA-2β. In 85-90% of individuals in which fasting hyperglycemia in detected at least one of these autoantibodies can be detected (7). Another helpful marker that increases before the clinical manifestations of diabetes is HbA1C that can be used to detect disease before onset of complications such as diabetic ketoacidosis (DKA) (8). Complication of DKA can be the first presenting symptom of diabetes, especially in children and adolescents. Other common symptoms include polyuria, polydipsia, polyphagia and weight loss (7). Patients with type 1 diabetes are prone to other autoimmune diseases such as Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia (8). Considering diagnosis for type 1 diabetes it should be noted that HbA1c is of less sensitivity since the disease progression may be very rapid so fasting or stimulated blood glucose measurements are preferred. Treatment aim is to maintain glucose levels in the reference range and to achieve an optimal glycemic control, basal insulin for overnight and between meal control is needed while bolus doses of fast acting insulin analogues treat hyperglycemia after ingested carbohydrate loads (7).

1.1.3. Type 2 diabetes mellitus

Previously known as ”adult-onset” or ”non-insulin-dependent” diabetes mellitus is caused by insulin resistance and relative insulin deficiency. It accounts for 90-95% of all diabetic patients. The exact etiology is not known but it shows a strong genetic predisposition as well as a strong association with obesity (especially central obesity) which causes insulin resistance. The risk also increases with age along with prior hypertension and dyslipidemia (5). A wide variety of modifiable lifestyle factors such as physical inactivity, smoking, alcohol consumption are also of great importance. A diet low in fibers and high in fat along with glycemic index are also positively associated with type 2 diabetes. Studies also showed that a moderate degree of gut microbial dysbiosis could lead to an increased risk (9). Type 2 diabetes is still under-diagnosed even though it is a common disease and there are different screening tests that are widely available. Microvascular disease is present in about 25% of individuals with newly diagnosed diabetes, indicating that they had had the condition for several years prior to diagnosis. This is believed to be due to lack of symptoms which is in correlation with endogenous insulin level that may be normal or even increased, depending on the speed of beta cell destruction. While most patients do not need insulin therapy throughout their life, some may need it early on in the disease course (5,10). Weight reduction has shown to increase insulin sensitivity although not completely restoring it to normal but in combination with oral

hyperglycemic therapy proved to be the best option. One of these drugs include the most commonly used - Metformin which has been proven to be effective in lowering glucose levels, increase insulin sensitivity, decrease hypoglycemia and the only drug that lowers macrovascular complications as well as reducing mortality in pateints with type 2 diabetes mellitus (9).

1.2 Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is defined as glucose intolerance that is detected at any time during pregnancy. This definition does not exclude the possibility of preexisting, undetected diabetes which has lead to appearance of new subcategories. The International Association of Diabetes and Pregnancy Study Groups (IADPSG) has divided it into two categories, preexisting diabetes ”overt diabetes” and diabetes detected in pregnancy ”gestational diabetes” while the WHO divides it into ”diabetes mellitus in pregnancy” and ”gestational diabetes” (11).

GDM is the most common complication of pregnancy and it affects around 1-20% of pregnant women worldwide. This number is increasing and is associated with the increasing number of type 2 diabetes mellitus cases. Another contributing factor to this increased number of cases is the fact that IADPSG has changed the criteria for diagnosing GDM. They recommend universal screening with glucose tolerance test (OGTT) and the diagnosis of GDM is set by only one glucose value above the cut-off value, which has in the past required two positive tests. GDM has a negative impact on both neonatal and maternal outcomes. By maintaining healthy blood glucose levels morbidity for both the mother and the infant decreases. Randomized controlled trials have shown that if screening and possible treatment of GDM at 24-28 weeks of gestation, it improves both maternal and perinatal outcomes (11).

1.2.1 Pathophysiology

During normal pregnancy there is a gradual increase of insulin resistance which leads to hyperinsulinemia that may predispose some women to develop diabetes. This insulin resistance begins in mid-pregnancy and continues during the third trimester (11). Thus if hyperglycemia is detected early in pregnancy (<24 weeks) it is more likely to be overt (12). Insulin secretion increases as the pancreas tries to compensate for the resistance, but when the pancreas is not able to produce high enough levels to overcome resistance, GDM develops.

Relative glucose intolerance develops when the pregnant women increase caloric intake, decrease physical activity and also the fact that there is an increase in maternal adipose deposition. In addition some of the possible causes includes placental hormones like adipokines, tumor necrosis factor alpha (TNFα), human placental lactogen, and human placental growth hormone. Other hormones that may also play a role are estrogen, progesterone and cortisol which all increase during pregnancy. The reason that GDM usually appears in late second or third trimester is that during pregnancy the insulin secretion and sensitivity varies. In the first trimester insulin secretion increases and sensitivity varies from decreased, normal to increased. From the mid pregnancy onwards sensitivity decreases, being worst at the late third trimester and usually disappears instantly post delivery of placenta (11).

There are many risk factors that are associated with development of GDM such as increased maternal age, obesity, persistent glucosuria, polycystic ovarian syndrome and hypertension. Regarding history, family history of diabetes, history of prevoius unexplained stillbirths and recurrent abortions are important risk factors (11).

1.2.2. Screening and diagnosing GDM

There is not an globally uniform strategy for screening and diagnosing GDM at this moment. In the 1960's O´Sullivan and Mahan proposed the first diagnostic criteria for GDM which was based on a three hour 100g OGTT in an attempt to try to identify women that had a high risk of developing diabetes after pregnancy. This original work was followed by many medical organizations but was later modified with the problem of frequent selection of different thresholds for diagnosing GDM. Nowadays two options are widely accepted: 1 step screening approach and 2 step Carpenter-Coustan screening approach as can be seen in table 1.

Table 1. Commonly used guidelines by different study groups for gestational diabetes mellitus (GDM) (11).

Criteria, year	Approach	Number of required abnormal value(s)	Fasting mmol/L (mg/dL)	One hour mmol/L (mg/dL)	2-hour mmol/L (mg/dL)	3-hour mmol/L (mg/dL)
IADPSG, 2010[3]	One-step, 75 g load	1	5.1 (92)	10.0 (180)	8.5 (153)	-
WHO, 1999[28]	One-step, 75 g load	1	7.0 (126)	-	7.8 (140)	-
WHO, 2013[4]	One-step, 75 g load (for diagnosis of GDM)	1	5.1 - 6.9 (92 - 125)	10.0 (180)	8.5-11.0 (153-199)	-
	One-step, 75 g load (for diagnosis of diabetes of pregnancy)	1	≥7.0 (126)	-	≥11.1 (200)	-
ACOG, 2001-2013[29]	2-step, 100 g load	2	5.3 (95)	10.0 (180)	8.6 (155)	7.8 (140)
CDA, 2003-2008[32]	2-step, 75 g load	2	5.3 (95)	10.6 (191)	8.9 (160)	-

IADPSG - International Association of Diabetes in Pregnancy Study Groups, WHO - World Health Organization, ACOG - American College of Obstetricians and Gynecologists, CDA - Canadian Diabetes Association

The 1 step approach was created by IADPSG and is preferred by ADA (American Diabetes Association). The recommendation is universal screening for all pregnant women. During the first antenatal visit it is recommended to screen for" pre-existing diabetes" or so called overt diabetes. Diagnosis can be established if any of the following criteria are met: fasting plasma glucose >7.0 mmol/L, casual plasma glucose of >11.1 mmol/L or if the HbA1c is >6.5%.

A second screening is recommended in 24-28 weeks of gestation if the results from the first screen were negative. The women should be fasting overnight for 8-14 hours prior to the 2 hour 75g OGTT and they should not change diet before the test. In order to set a diagnosis of GDM one or more values need to be equal to or exceed the thresholds; Fasting plasma glucose (FPG) 5.1 mmol/L, 1 hour plasma glucose 10mmol/L and 2 hour plasma glucose 8.5mmol/L. The diagnosis of GDM can also be made if the FPG is between 5-7 mmol/L during any time of pregnancy (11). Milder cases of hyperglycemia can be identified and diagnosed as GDM with the 1 step approach. Diagnosis and treatment of such mild, early identified diabetes has not shown a clear benefit even though there is a clear association between maternal hyperglycemia and both maternal and perinatal outcomes (13).

The 2 step Carpenter-Coustan screening approach is recommended by the American College of Obstetricians and Gynecologists (ACOG) and National Institute of Health. In this approach the first screening is done at week 24-28 of gestation by 50 g oral glucose challenge test (GCT), without the need of prior fasting. The plasma glucose levels are then measured after one hour and the threshold is ≥7.2, or ≥7.8 mmol/L. Most women don't need further testing however approximately 20% exceed the tolerated plasma glucose level in the first screening and therefore need to proceed to the three hour fasting 100g OGTT diagnostic (11,13). The most sensitive strategy, according to the ACOG, is universal screening, but some pregnant

women with low risk may be less likely to benefit from the procedure. Low risk pregnancy is considered to be: women <25 years, not overweight, no family history of diabetes in first-degree relatives, no history of abnormal glucose tolerance or adverse pregnancy outcomes and not being a member of a racial or ethnic group with a high prevalence of diabetes (14).

In 2013 The National Institutes of Health recommended to compere these two approaches with a randomized trial in respect to clinically important outcomes (13). By the year 2014 ADA readdressed both approaches and their recommendations with no clear conclusion of with strategy was superior to the other (11). WHO has applied the 1 step approach as their recommendation for screening of GDM with the goal to minimize the adverse outcomes associated with maternal hyperglycemia (15). The 1 step approach has a lower threshold for diagnosing GDM but the compliance to the testing is lower since women have to be fasting in order to correctly make a diagnosis. The 2 step approach is an easier choice for the patient and thereby more compliance can be expected but the threshold for diagnosis is higher. In conclusion more women will be likely to be diagnosed with GDM by the 1 step approach than 2 step approach, whereas more women will adhere to step 2 approach. In 2021 a pragmatic randomized clinical trial was performed in which 23,792 women were included where the researchers tried to see which approach would be the better option. It showed that 16,5% of women in the step 1 approach were diagnosed with GDM while only 8,5% were diagnosed with GDM with the 2 step approach. However no significant difference in maternal or perinatal outcomes could be proven (13). The same results were conducted by another study done earlier in 2014 (16).

1.2.3 Treatment of gestational diabetes mellitus

Primary strategy for managing GDM is life-style modification and it is suggested that over 80% of pregnant women can control their hyperglycemia with medical nutrition therapy, physical activity, and weight management alone. An even higher percentage can be reached if the IADPSG diagnostic thresholds are used (17). Numerous randomized controlled trials (RCTs) indicate that it is particularly true when interventions are initiated in the first or early second trimester. The study of the perinatal data showed that the diet-controlled GDM group's placental and fetal weights were significantly lower than those of other diabetic patients and of pregnant women with uncomplicated pregnancies (18).

To achieve glycemic control in both gestational diabetes mellitus and preexisting diabetes in pregnancy, fasting and postprandial self-monitoring of blood glucose are advised.

ADA recommends glucose levels as following: fasting <5.3 mmol/L, one hour post prandial <7.8 mmol/L and two-hour postprandial <6.7mmol/L. Postprandial monitoring is related to better glucose control and a reduced risk of preeclampsia. Women with preexisting diabetes are sometimes also advised to check preprandial glucose.

Lower glucose levels in pregnancy is physiologic due to insulin-independent glucose uptake by the fetus and placenta in addition to higher erythrocyte turnover. This leads to lower HbA1c level causing the target to be around 6% if hypoglycemia can be avoided. The HbA1c should therefore be used as a secondary measure. However in second and third trimester effect of erythrocyte turnover is smaller and studies show that a HbA1c <6% has the lowest risk of large-for-gestational-age infants, preterm delivery, and preeclampsia (17).

Fetal risk for macrosomia increases linearly with maternal glucose level, with excessive maternal weight doubling the risk. Weight gain alone poses a significantly high risk for fetal macrosomia even when glucose levels are within normal limits (19). Even though little evidence exist on specific nutritional approaches small weight reduction improves glucose levels significantly, but it should be used with caution in pregnancy because severe caloric restriction with weight loss may in turn lead to ketonemia and small-for-gestational-age infants (20). Several studies on the impact of caloric restriction in obese women for the control of GDM were published between 1985 and 2000. Due to its unfavorable impact on raising plasma ketone bodies, caloric restriction has been a controversial for many years. The majority of studies and literature reviews found that in obese women with GDM, a modest caloric restriction of 30–33% of total energy has a positive impact on glucose metabolism without resulting in ketoacidosis and leading to improved glycaemic control and thereby decreasing poor pregnancy outcomes. Another important component proven to be beneficial for glycemic control was physical activity in addition to nutritional adjustments. ADA and ASN (American Society for Nutrition) states that nutrition counseling could be considered before, during and after pregnancy describing the role of diet and physical activity in reproductive health (21).

An individualized nutrition plan should be made with a adequate caloric intake. The DRI (daily recommended intake) recommend a minimum of 28 g fiber, 71 g protein, 175g of carbohydrates for all pregnant women. The amount and type of carbohydrates will impact the blood glucose concentration (17). Studies are conflicting considering not only carbohydrate but also fat intake. Some studies claim that carbohydrate restriction improves glycemic control, reduces the requirement for insulin therapy, lowers the incidence of LGA newborns, and lowers the need for cesarean sections for cephalopelvic disproportion and macrosomia (21). While other state that a lower carbohydrate intake lead to a higher fat diet and thereby increases the

risk for GDM (22,23). On one hand studies in which women were consuming more complex carbohydrates and less fat showed a decrease in maternal FFA (free fatty acids) which are responsible for insulin resistance and have also been correlated with excessive fetal growth. By decreasing FFA an increase in insulin sensitivity and better glycemic control was seen without the need of medication (23).

On the other hand studies on short-term interventions have found that diets promoting high monounsaturated fat intake have better glycemic control versus diets high in carbohydrates. While population-based studies have found that consuming monounsaturated fat has either no effect or a negative impact on markers of insulin action and glycemic control. Conclusion was drawn that type and mode of consumption of monounsaturated fats differed and were therefore responsible for the conflicting results. The most important findings were that gestational diabetes and glucose intolerance were unexpectedly and independently associated with lower intakes of polyunsaturated fats. A higher consumption of polyunsaturated fats seemed to offer protection from glucose intolerance and GDM (24).

Diets that have been proven to be beneficial in reducing hyperglycemia and thus risk for GDM include Mediterranean diet, DASH diet, AHEI-2010 diet and Western and prudent diet. AHEI-2010 diet seems to be the best choice according to one study that was conducted in 2022. This diet places a focus on increasing consumption of legumes and nuts, cereals, fruits and vegetables, omega-3 fats, and polyunsaturated fatty acids while reducing consumption of red and processed meats, sodium, sugary drinks, and alcohol. Risk reduction was 19–46% and when combined with other risk factor reductions such as stop smoking, normal body weight and physical activity the percentage reached 83%. Exercise was only effective if started in first trimester and only mild-to moderate intensity while vigorous exercise was ineffective. This meta-analysis showed that diet had a significant effect of prevention of gestational diabetes but exercise showed only limited benefits in from of prevention (25).

The cornerstone of gestational diabetes management is control of blood glucose by lifestyle measures, such as regular exercise and medical nutrition therapy, are the first line of treatment for GDM (11). Studies suggest different success rate between 70-90% hyperglycemic control by only lifestyle modification (14,26). To ensure that the glycemic targets are met, patients should frequently check their blood glucose levels at home. If glycemic control is not achieved by these measures, medical therapy should be started (11). Up to 30% of GDM patients will require pharmacotherapy to maintain a healthy glycemic level. Insulin has been proven to be the safest choice in pregnancy because it is a large molecule that is not able to cross placenta and is therefore recommended as first line by both ADA and ACOG. The

patient's body weight, gestational age, and the time of day that hyperglycemia is present all affect the dosage and timing of administration (26). In the past only regular and inter-mediate acting NPH insulin was used but nowadays rapid acting lispro and aspart are also available (11). However the data is conflicting regarding which type of insulin is the best choice. Some suggest rapid acting insulin have less risk of hypoglycemia and better control of one hour post prandial glucose concentration while other state the opposite, in addition stating that lispro has been associated with macrosomia of the fetus (14,26).

Oral hypoglycemic agents are less expensive and have better patient compliance (11). The most commonly used oral agents can be divided into two classes, sulfonylureas - glyburide and biguanides - metformin. The former stimulate insulin production and release by pancreas while the latter stimulate insulin sensitivity of tissues (14). Comparing the two oral medications, metformin seems to be a safer option than glyburide because glyburide has been found in umbilical cord samples in very high concentration which can be an explanation for neonatal hypoglycemia in addition to higher birthweight in babies born to these mothers (26). Although metformin has been measured in high concentrations in the umbilical cord as well, no adverse outcomes of fetus has been reported except of preterm labour (14,26). Moreover metformin was associated with lower rates of neonatal hypoglycemia than insulin and women were also more likely to use metformin than insulin. In about half of the cases metformin alone is not sufficient to control hyperglycemia and an addition of insulin may be needed (11).

1.2.4. Complications of gestational diabetes mellitus

It is of out most importance to treat gestational diabetes mellitus, otherwise it may lead to many severe complication for both mother and child. These complications have been demonstrated by the HAPO (Hyperglycemia and Adverse Pregnancy Outcomes) study in which maternal glucose levels were greatly associated with adverse pregnancy outcomes. Primary complications included cesarean delivery, birth weight >90th percentile, clinical neonatal hypoglycemia, and fetal hyperinsulinemia (26). The secondary outcomes proven by the same study included preterm birth, shoulder dystocia and/or birth injury, admission for neonatal intensive care, hyperbilirubinemia and pre-eclampsia among others (27). GDM has been linked to significant short- and long-term health effects, including a higher chance of developing cardio-metabolic illnesses later in life in both women and their offspring. A sevenfold increased risk for development of DM2 has been shown, especially in developing countries with the lowest prevalence in Europe (28). In the first nine months following delivery, the rate of

diabetes development was rapid, but remained relatively constant thereafter (29). Women who have had GDM also have higher risk of developing premature cardiovascular and renal diseases which can be explained by the increase in lipid concentration and higher blood pressure in this group (30). The offspring also has an increased risk of developing metabolic syndrome and DM2 at a younger age (31).

1.3 GLUT transporters

There are fourteen GLUT proteins found in humans that are encoded by the SLC2 genes and they form membrane transporters, which can be seen in figure 1. GLUT 1-5 have been studied thoroughly and their roles as glucose and/or fructose transporters has been well established, whereas the rest of the transporters roles remain uncertain. Every cell in the human body have at least one, and usually several GLUT transporters in their membrane (32). GLUT 1-4 facilitate passive movement of glucose down concentration gradient, mostly from the blood into the cell but also from the cell into the bloodstream, especially in the liver. This process maintains a relatively constant blood glucose by moving glucose in the the direction it is needed for cell metabolism, making the transporters often rate limiting (33).

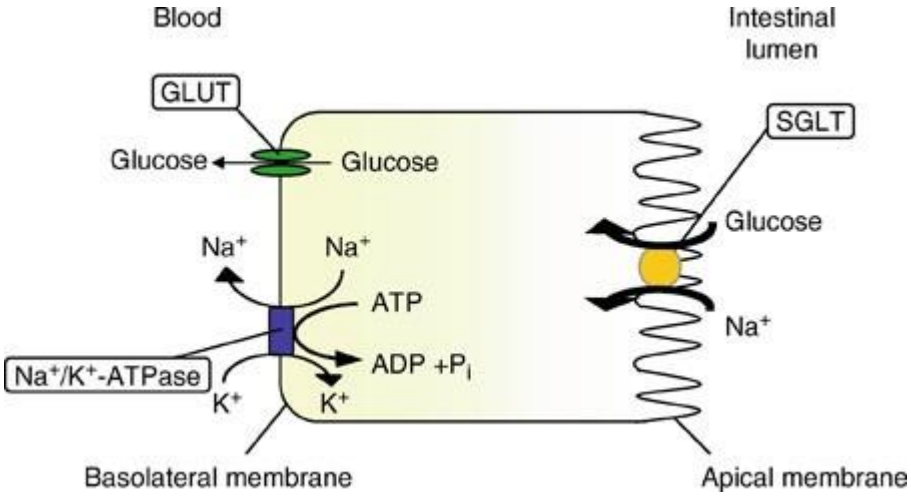

Figure 1. Glucose transporters (34).

GLUT-1 was the first one to be discovered in 1985 and is the one which has been studied most extensively (32,35). It mostly transports glucose and is primarily expressed in human erythrocyte membrane, but is also found in brain endothelial cell in which it plays a critical role

of cerebral glucose uptake (32). Studies show that rapid efflux of glucose from erythrocytes happens when plasma glucose concentration falls (33). Activity of GLUT-1 may be acutely upregulated which can be useful to oppose the effect of arterial blockage that causes strokes and myocardial infarctions but less so when upregulated during oncogenesis (35).

GLUT-2 is characterized by having a low affinity for glucose and is the the major glucose transporter of hepatocytes.

GLUT-3 has higher affinity for glucose and is together with GLUT-1 an important transporter in the brain. In human white blood cells GLUT-3 is confined to intracellular vesicles but during proliferative stimuli it translocates to the cell membrane.

Similarly GLUT-4 migrates to the cell membrane of adipocytes, skeletal muscles and cardiomyocytes but in response to insulin, thus preventing chronic hyperglycemia. A defect of this process causes peripheral insulin resistance which contribute to the development of type 2 diabetes.

GLUT-5 was initially found in human small intestine and it has been shown to have high specificity for dietary fructose which it transports across the apical membrane.

GLUT-8 is entirely intracellular and it plays an important role in spermatogenesis.

GLUT-9 has two different variants, GLUT-9a and GLUT-9b. GLUT-9a is expressed in the liver, kidney, intestine, leukocytes, and chondrocytes whereas GLUT-9b is only expressed in the kidney and liver. It has been established as a urate transporter and not a glucose/fructose transporter as initially believed. Mutation of the SLC2A9 gene that encodes GLUT-9 transporter causes large excretion of urate which often is associated with nephropathy and renal uric acid crystals which characterizes the Dalmatian dog (32). Inactivation of liver specific GLUT-9 gene leads to hyperuricemia without other physiological changes. Epidemiological studies suggest that an elevated plasma uric acid level may lead to hypertension, atherosclerosis and even insulin resistance (35). It has been reported that there is an exchange of urate for glucose and fructose in the kidneys, suggesting that loss of urate may lead to glucose reabsorption (33).

The SLC2A13 gene encodes a H+/myo-inositol co-transporter called HMIT, which has no glucose transport properties. It is expressed predominantly in the brain, found both in glial cells and neurons. In neurons it works as GLUT-3 and GLUT-4, present intracellularly and migrates to the membrane to increase myo-inositol uptake, triggered by neuronal activation, activation of protein kinase C or Ca++ influx (36). Variants of HMIT transporter is believed to be associated with mood disorders such as bipolar disorder since the HMIT targeting drug LiCl has shown effect (35). Sequencing of the human genome lead to the later discovery of GLUT

6, 7, 10, 11, 12 and 14 so they are less studied and therefore little is known about their specific functions and roles (32,35).

1.4 Placenta

Placenta is essential for reproduction in mammals including humans. It is a complex, highly specialized organ formed during pregnancy to sustain growth and development of the fetus (37). There are many different shapes and sizes of placentas but it can roughly be described as a discoid organ with an average diameter of 22cm, 2.5 cm thick at the center with a wight of about 500g (38). During the first trimester the placenta grows faster than the fetus but approximately at 17 postmenstrual week the weights are equal (12).

1.4.1 Development of placenta

The first cell lineage to segregate is the so called trophoblasts that form the external epithelial layer of the placenta. These cells are pluripotent stem-cells and the name comes from ”tropho” meaning nourishment and ”blast” meaning embryonic, named by Hubrecht in 1904 (37). Trophoblasts differentiate into two cell lineages, villous and extravillous. Villous trophoblasts form the outer epithelial layer of the chorionic villi by fusion and formation of the outer multinucleated syncytiotrophoblast and inner mononucleated cytotrophoblasts. The extravillous trophoblasts are responsible for establishing blood flow to the placenta by invading spiral arteries in the decidua all the way to the inner third of the myometrium. Invasion of spiral arteries is carried out by two different types of extravillous trophoblasts - the penetrating endovascular trophoblasts and the interstitial trophoblasts that surrounds the arteries and prepares them for endovascular invasion. Only decidual spiral arteries are invaded, not the decidual veins (39). Uterine natural killer cells (uNK) has also been shown to play an important part of this invasion. Unlike natural killer cells these cells helps not only with the migration of extravillous trophoblasts through the endometrium and myometrium, but also releases cytokines and growth factors that converts the spiral arteries to low-resistance and dilated vessels. This conversion secures consistent maternal blood flow into the intervillous space so it becomes available to the fetus via chorionic villi. Interaction between maternal immune system and placental cells appear to play an physiological role rather than classic immunological role (12,37).

1.4.2. Function of placenta

Its main function is exchange of gases, nutrients and waste products between maternal and fetal circulations, acting as fetal lungs, kidneys, gut and liver (37,39,40). The endocrine function of the placenta is another important entity that regulates maternal physiology and metabolism that is essential for a successful pregnancy. It secretes more than 100 peptides and hormones such as placental lactogens that raises concentration of nutrients in the maternal blood so that they are readily available for the growing fetus and also later for milk production (40). Another hormone that is essential for maintainance of pregnancy is the Beta-Human Chorionic Gonadotropins (β-hCG) that is secreted by the trophoblasts and acts to maintain corpus lutheum in order to continue producing progesterone in order to maintain pregnancy (12). During the first trimester the rate of growth of the fetus depends on the endometrial gland secretions and is fairly constant between individuals. These secretions are rich in carbohydrates and lipid droplets as well as cytokines and growth factors but their complete composition is still not well known. Thus the secretions are thought to play an important role in placental proliferation and differentiation regulation in early pregnancy. Low oxygen concentration that is present in early the first trimester before involvement of spiral arteries appears to have a protective effect, namely it protects from teratogenesis mediated by reactive oxygen species. Free radicals can cause a direct attack on genomic DNA disrupt embryonal development, initiating congenital abnormalities (41). This low oxygen concentration is enabled by endovascular trophoblasts that forms plugs in the spiral arteries (42).

1.4.3 Morphology of placenta

The placenta is made up of a fetal part and a maternal part. A schematic drawing can be seen below in figure 2. Maternal part of placenta comes from the modified endometrium called decidua which consists of three parts decidua basalis, capsularis and parietalis(12,39,43). In the decidua endometrial arteries form the so called spiral arteries that have a muscular wall and a narrow lumen. Chorionic plate belongs to the fetal part of the placenta to which the umbilical cord is attached. Chorionic villi arise from the chorionic plate and they form a tree-like structure that is enveloped in the placental membrane(38,43). Placental membrane is formed by two separate units – placental villous membrane (syncytioplasm) and fetal capillaries that together form the so called vasculosyncytial membrane (VSM) which functions as one unit. The maintenance of exchange surface area and efficient diffusion distance of fetomaternal surfaces

are the most important characteristics of the VSM (44). Chorionic vili are located within the intervillous space which separates maternal part of placenta from the fetal part. In this space exchange of nutrients and gases occurs over the VSM which acts as prevention of mixing fetal and maternal blood (38,45).

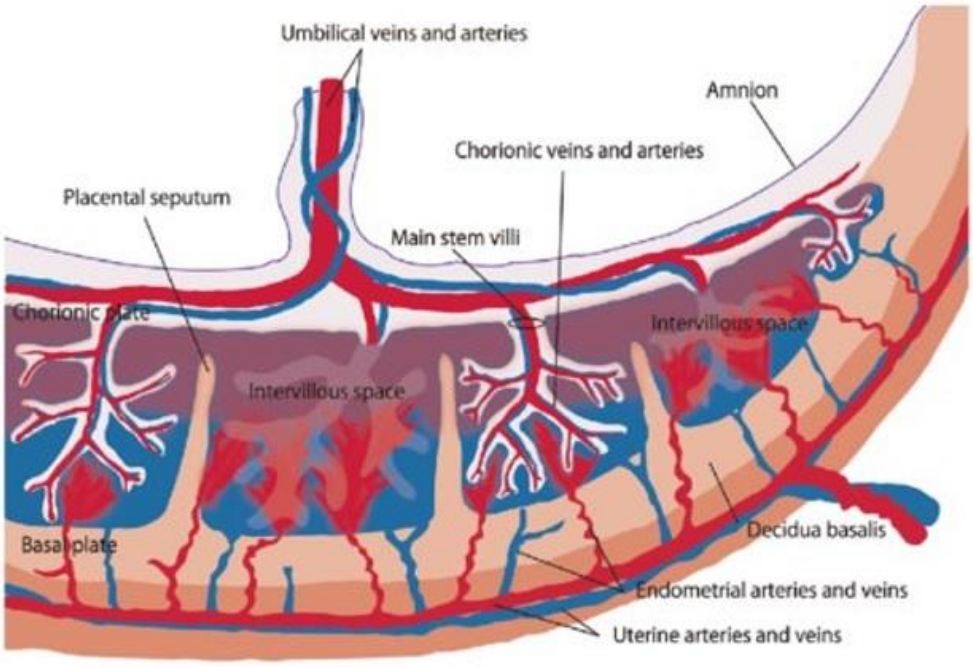

Figure 2. Schematic drawing of the placenta (cross-sectional image) (46).

1.5 GLUT transporters in placenta

Fetal growth is maximal during third trimester and thereby also glucose demand (47). The developing fetus is unable to produce endogenous glucose and is therefore dependent on maternal supplies, transported through placenta via the GLUT transporters (48). There are 14 GLUT isoforms identified and three (GLUT-1, GLUT-4, GLUT-9) seem to be of particular importance for the placental glucose exchange. GLUT-1 is the primary and most abundant glucose transporter of placenta found in cyto and syncytiotrophoblast of the placental villi (18). Its expression increases during pregnancy and the maximal level is reached in the third trimester and is the only transporter present in the syncytium as a functional protein near term. A three times higher number of the GLUT-1 transporters are located on the maternal side of the syncytiotrophoblast (MVM) compared to the fetal side (BM) and is believed to have a rate limiting effect of glucose transport between mother and fetus, thus protecting the fetus from hyperglycemia. However studies show that with hyperglycemia in diabetic patients these

transporters up regulate and thereby loose the protective effect (48). Another interesting factor influencing enhanced expression of GLUT-1 seems to be increasing maternal age (49).

While some studies suggest that the insulin-dependent GLUT-4 located intracellularly in stromal cells of the placental villi pose an important role of whole-body as well as placental glucose homestasis, other strongly disagree stating its expression is extremely low and thereby not contribute significantly to the glucose uptake in the placenta (18,47). The same goes for the expression of the transporter by insulin. One states there is an increased expression of this transporter may be found in diabetic mothers on insulin therapy. The other that it is not subject to regulation by insulin. GLUT-9 is the least known among all isoforms capable of glucose and fructose transfer, present in the placental syncytiotrophoblast and vascular endothelium.

Changes in expression and activity of GLUT transporters in the placenta can be observed in gestational diabetes mellitus (GDM) and pre-gestational diabetes mellitus (PGDM). Consequences of these changes have been speculated to increase glucose concentration in the fetal circulation which leads to elevated production of growth factors and finally macrosomia. This is especially true for GLUT-4 in women treated with insulin. However some studies have shown opposite results with a decrease of GLUT-4 transporters in response to insulin treatment which corresponds to the fact that the number of insulin receptors in the syncytium decreases as pregnancy progresses (18). On the other hand some studies show no effect on expression of GLUT-4 in glucose transport across the placenta in the presence of insulin (47). Expression of GLUT transporters are also influenced by insulin-like growth factor 1 (IGF-1), corticotropin releasing hormone (CRH), glucocorticoids and hypoxia depending on gestational age and concentration. For example glucocorticoids downregulate GLUT-1 and GLUT-3 whereas CRH increase GLUT-1 but decrease GLUT-3 (48). In conclusion many of the existing studies have been done in vitro which may show importance of every parameter on its own, in vivo all these elements interact and may hence show contradictory results (47,48).

1.6 GLUT-9 transporter in placenta

GLUT-9 was discovered in the year 2000 but little progress has been made toward understanding the mechanism of its action (50). It can be found in placental syncytium and vascular endothelium and it functions as both a glucose and a fructose transporter. It has been shown that insulin-controlled GDM and pregestational GDM women's placental samples exhibit significantly higher levels of GLUT-9 expression. In the membrane fractions of the syncytiotrophoblast in diabetic placentas, there are two distinct GLUT-9 isoforms: GLUT-9a

and GLUT-9b. GLUT-9a was significantly elevated in syncytial BM in all diabetic pregnancies whereas GLUT-9b was found in both MVM and BM only in women with insulin dependent gestational diabetes and pregestational diabetes mellitus. Thus expression of GLUT-9 was increased in diabetic women, notably during insulin therapy. Blood fructose levels in diabetic patients are known to be raised, but fetal and umbilical cord blood fructose concentrations are higher when compered with maternal levels which may be damaging to the fetus. It may result from both the endogenous generation of fructose by the fetal-placental unit during healthy pregnancy utilizing glucose as the substrate and -as was proven by animal studies - the reaction to an excessive maternal fructose consumption. In the animal models administration of fructose solution resulted in hyperinsulinemia leading to significantly higher birthweight in the offspring believed to be caused by an increased GLUT-9 expression in the placenta. However, research on transplacental fructose transfer and how it affects a fetus's growth in humans is lacking. It should be emphasized that other fructose transporters, such as the main GLUT-5, have not yet been identified in the placenta (18). One human study has been done where a 40% fructose solution was administered to the pregnant women just before delivery trying to depict diabetic conditions. The umbilical cord fructose level increased 3-fold in comparason to maternal level which is supporting the hypothesis of increased GLUT-9 transporters in placenta. It has been demonstrated that fructose contributes to insulin resistance, thus play a role in the metabolic syndrome's effects. It has also been demonstrated that elevated fructose reduces glyceraldehyde 3-phosphate dehydrogenase, which in turn increases the formation of ROS (GAPDH). Although speculative, it's likely that elevated fructose levels in gestation and enhanced ROS generation inside the placental or fetal compartment are caused by increased transport of fructose via GLUT9 in the placenta. In this way, a rise in GLUT9 during pregnancy may be linked to diabetic complications (51).

2. OBJECTIVE

Aim: Primary outcome was to determine GLUT-9 immunohistochemical expression in placentas from pregnancy complicated with gestatinal diabetus mellitus and to compare them to placentas from normal pregnancies. Furthermore we aim to invastigate GLUT9 immunohistochemical expression for each placental component seperately, including decidual cells (DC), villous trophoblast cells (VTB), extravillouos trophoblast cells (EVTB) and vasculosyntitial membranes (VSM) and to compared the results between studied placental groups. Secondary outcome was to compare neonatal birth weight and placental weight between study groups.

Hypothesis: We expect a higher GLUT-9 immunohistochemical expression in all studied placental components in placentas from pregnancies complicated with gestational diabetes mellitus in comparison to placentas from normal pregnancies. Likewise, we expect a neonatal birth weight, as well as placental weight to be higher in gestational diabetes mellitus group in comparison to normal pregnancy group.

3. MATERIAL AND METHODS

This study is a case-control study that was conducted in Pathology department at the University Hospital Centre Split and Department of Obstetrics and Gynecology of the same hospital in the period January 1st, 2019 – December 31st, 2019.

The placental samples were obtained from 24 women after vaginal or cesarean delivery at the Department of Obstetrics and Gynecology, Split University Hospital Centre. The inclusion criteria were as follows: maternal age >18, singleton pregnancy, and gestational age of >37 weeks, diagnosis of GDM based on the criteria mentioned below for the GDM group. Exclusion criteria were fetal malformations, intrauterine fetal growth restriction, maternal chronic or pregnancy-induced hypertension, maternal or fetal inflammatory response, as well as the diagnosis of GDM for the control placental group.

The placentas were divided into two groups: study group of women with diet regulated GDM (13 placentas) and control group (11 placentas) of women with an uncomplicated pregnancy. GDM was diagnosed based on the 75 g oral glucose tolerance test (OGTT) performed between 24 and 28 gestational weeks, in accordance with the criteria defined by the HAPO study (26). Placentas were collected within 20 minutes of delivery, fixed in 10% buffered formalin and sent to Pathology Department for further analysis. For the purpose of the study, one full-thickness placental section was taken from macroscopically normal placental disc, close to the umbilical cord insertion. All placental samples were examined by the two perinatal pathologists blinded to the assigned clinical category.

Immunostaining was performed on the same serial section of each placental sample, as follows: paraffin sections were mounted on super frost slides (Thermoscientific, Dreieich, Germany) and processed in an automatic stainer (Ventana Bench Mark Ultra autostainer, VentanaRoche, Tucson, AZ). For detection of GLUT-9 primary ‘‘ready-to-use’’ polyclonal rabbit antibody (ab104623, Abcam, USA, dilution 1:100) was applied. UltraView Universal DAB Detection Kit (RRID:AB_2753116, Ventana, Tucson, Arisona, USA) was used as secondary antibody. Brown staining of the cell membrane and/or cytoplasm was considered as positive (Figure 1A, 1B and 1C). Endothelial cells of fetal blood vessels within chorionic villi served as a positive control. Expression of GLUT-9 was determined separately for DC, VTB, EVTB and VSM by the HSCORE method using the equation: HSCORE = Pi(i+1), where i is the intensity of staining, with a value of 1 (weak), 2 (moderate) or 3 (strong), and Pi the percentage of stained trophoblast cells of each intensity (6). All measurements were performed manually using a ×40 objective placental sample was analyzed throughout 10 fields of HPF.

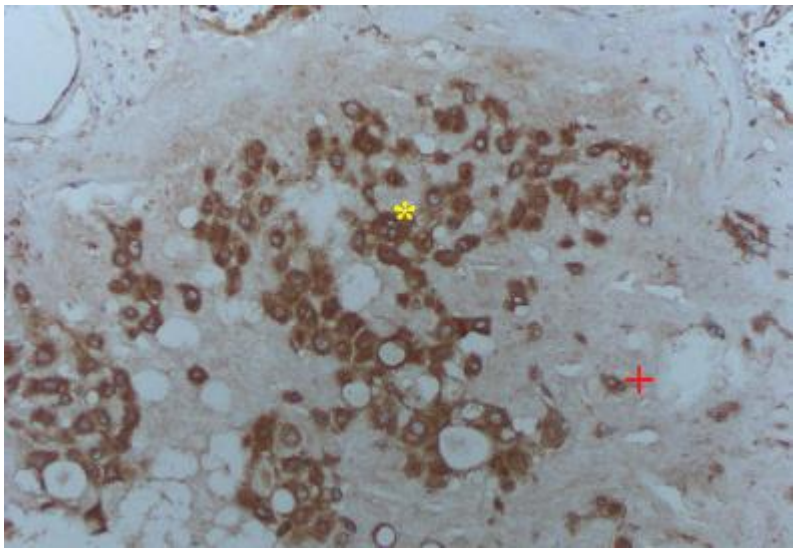

Figure 3. GLUT-9 immunohistochemical expression in extravillous trophoblast (yellow mark) and decidual cells (red mark) of placentas from pregnancies complicated with gestational diabetes mellitus (Magnification 200x, Olympus Image Analyzer).

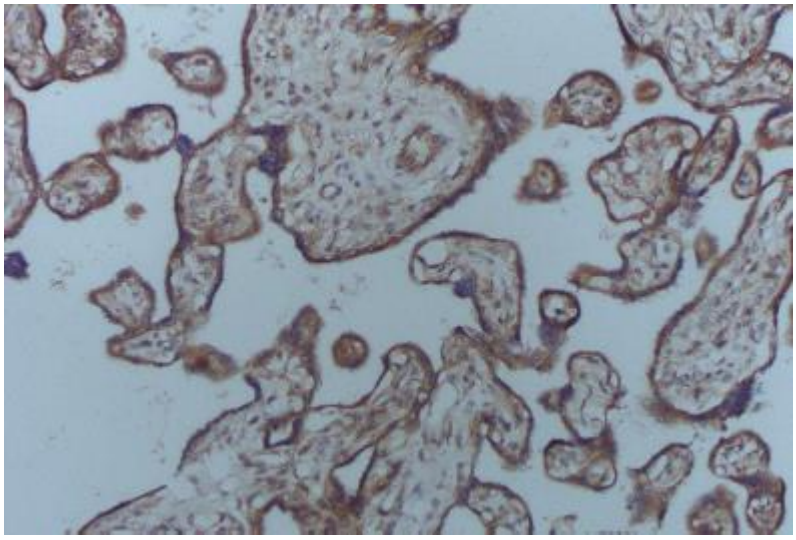

Figure 4. GLUT-9 immunohistochemical expression in chorionic villi of placentas from pregnancies complicated with gestational diabetes mellitus (Magnification 100x, Olympus Image Analyzer).

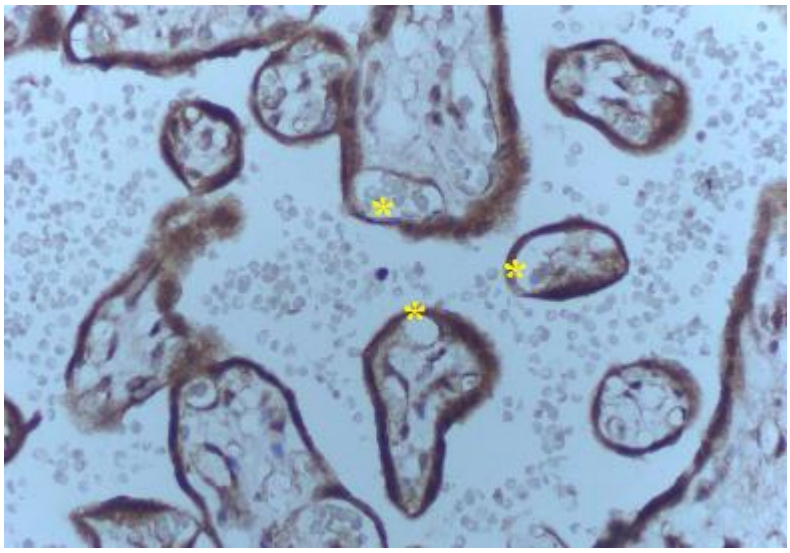

Figure 5. GLUT-9 immunohistochemical expression chorionic villi with emphasis on vasculosyntitial membranes (yellow mark) in placentas from pregnancies complicated with gestational diabetes mellitus (Magnification 400x, Olympus Image Analyzer).

Data distribution was estimated using Kolmogorov-Smirnov test. To interpret the statistical significance Mann-Whitney U test and $\chi 2$-square test were used. Data is presented as a median with minimum and maximum values, or frequencies. The statistical significant value was set at $P<0.05$, and statistical analyses was performed using MedCalc software (MedCalc software, Ostend, Belgium).

All procedures in presented study were in accordance with ethical standards of the institution and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Ethics Review Board of University Hospital of Split with reference No. 2181-147-01/06M.S.-20-2

4. RESULTS

This study included 24 placentas from both vaginal delivery and caesarean section. Out of these 24 placentas, 13 (54%) were from women with diet-regulated GDM while 11 (46%) were from the control group consisting of women with an uncomplicated pregnancy.

Women with GDM were a bit older in average than the control group (Z=1,565; *P*=0,118), this was not statistically significant. There was no difference in gestational age between the groups (Z=0,148; *P*=0,882). Both fetal birth weight (Z=1,448; *P*=0,148), and placental weight was a little higher (Z=0,782; *P*=0,434) in the GDM group although not statistically significant. Considering gender, the ratio male/female was higher in the GDM group than the control, this finding showed no statistical significance (χ2=2,701; DF=1; *P*=0,100) (Table 2)

Table 2. Demographic characteristic of studied patients from gestatinal diabetes mellitus grup (GDM) and from normal pregnancies (control group)

	GDM N=13	Control group N=11	*P*
Maternal age (years)	33 (22-43)	30 (20-36)	0.118
Gestatonal age (weeks)	38 (37-41)	38 (37-40)	0.882
Fetal weight (g)	3670 (2660-5140)	3350 (2210-3710)	0.148
Gender (male/female)	5/8	3/8	0.100
Placental weight (g)	587 (428-902)	537 (413-620)	0.434

*Mann-Whitney U test, presented as median with highest and lowest values
**Chi-square test

Expression of GLUT-9 was determined separately for DC, VTB, EVTB and VSM by the HSCORE method described previously. There was no statistically significant difference in GLUT-9 immunohistochemical expression in DC (Z=0,580; *P*=0,562) nor in EVTB (Z=0,580; *P*=0,562) between the groups. Expression of GLUT-9 in VTB was a little bit higher in the control group but without statistical significance (Z=1,527; *P*=0,127). Similar results were shown in VSM but also these were of no significance (Z=1,122; *P*=0,262) (Table 3).

Table 3. HSCORE of GLUT-9 immunohistochemical expression in placentas from pregnancies complicated with gestational diabets mellitus (GDM) and placnetas from normal pregnancies (control group)

	GDM N=13	Control group N=11	*P*
Decidual Cells (DC)	4 (3,4-4)	4 (3,5-4)	0.562
Extravillous trophoblasts (EVTB)	4 (3,4-4)	4 (3,5-4)	0.562
Villous trophoblasts (VTB)	3,4 (2,4-4)	3,9 (3,1-4)	0.127
Vasculosyntitial membrane (VSM)	3,6 (2,4-4)	3,9 (3,1-4)	0.262

*Mann-Whitney U test, presented as median with highest and lowest values
**Chi-square test
DC - Decidual cells
EVTB - Extravillous trophoblasts
VTB - Villous trophoblasts
VSM - Vasculosyntitial membrane

5. DISCUSSION

In this study we investigated the immunohistochemical expression of GLUT-9 in placentas from women with diagnosed GDM. Previous studies have demonstrated presence of GLUT9 in human placentas, mainly on the syncytiotrophoblasts (STB) and vasculosyncytial membrane (VSM), however data on the GLUT9 in other placental compartments is insufficient (18). Therefore we set to investigate GLUT9 immunohistochemical expression in all of the placental compartment including DC, VTB, EVTB and VSC in GDM placentas and to compare them with placentas from normal pregnancies. In our study we could not prove an increased GLUT-9 expression in any of the above mentioned compartments of placenta in the GDM group compared with the healthy control. However an interesting finding was that there was a slight increase of GLUT-9 expression in VTB and VSM in the control group compared to GDM, although statistically insignificant. There is very limited research on the topic of GLUT-9 transporter in general, especially its effects in placentas. The available research that exist is mainly focused on its expression in other tissues like the kidney and liver. We managed to find one study preformed by Stanirowski et al where GLUT-1, GLUT-4 and GLUT-9 expression was investigated in the placenta. They studied a diabetic group that was further subdivided into diet-controlled gestational diabetes mellitus (GDMG1), insulin-controlled gestational diabetes mellitus (GDMG2), pre-gestational diabetes mellitus (PGDM) and compared them to a healthy control group. Morphometric analysis revealed a significant increase in the expression of GLUT-4 and GLUT-9 in insulin-dependent diabetic women (GDMG2 + PGDM) as compared to both, control and GDMG1 groups. In this study two isoforms were described GLUT-9a and GLUT-9b that was shown to have different impact on placenta in GDM. The former could be found in a larger scale in all diabetic pregnancies while the latter was associated with insulin-dependent diabetic pregnancies (18).

A major difference between our study and the study by Stanirowski et al is the patient sample, where we only investigated diet-controlled GDM and compared it to a healthy control, while they had four groups in total. Another major difference is that in our study we did not examine the two above mentioned isoformes GLUT-9a and GLUT-9b separately so that may also have affected our results along with the fact that women in our study were not insulin dependent so elevated expression of GLUT-9 could not be proven. Unlike Stanirowski et al who used morphometric software to determine immunostainings, we used HSCORE method using the equation: HSCORE = Pi(i+1). This was carried out twice by two independent perinatal pathologists. Expression of GLUT-9 was determined separately for decidual cells,

villous trophoblast, extravillous trophoblast and vasculosyntitial membranes as nowhere else before to our knowledge. Even though our studies have some differences we found no statistically significant difference between the diet controlled GDM and the healthy controls and these findings are in accordance with the research from Stanirowski et al (18).

In conclusion we can presume that GLUT-9 takes no significant part in placental glucose uptake if there is no need for insulin treatment of GDM and there is a high possibility that it can be treated only by diet as Stanirowski et al described (18). Since 1 step approach has a lower threshold for diagnosing GDM, appropriate interventions can be initiated in an earlier stage and thereby prevent severe complications and also the need for insulin therapy. With a proper follow up including regular glucose measurements and education of the pregnant women raising awareness of the condition and its effects on the pregnancy outcomes, their compliance of a strict diet may increase and thus prevent some of these outcomes. Earlier termination of pregnancy may also play an important role of minimizing the risks of macrosmia, higher placental weight and the need for C-section. Proper follow up, diet control and earlier pregnancy termination minimize risks of macrosomy, higher placental weight and increased expression of GLUT-9 in diet regulated GDM.

Many studies showed a strong correlation between maternal gestational diabetes mellitus and an elevated newborn birth weight and a larger placental mass. This can largely be explained by the fact that it has been proven that placentas in women with GDM have a higher expression of GLUT transporters in their membranes, mainly in the syncytiotrophoblasts (52). In addition the syncytiotrophoblasts were also shown to be hypervascularized with an increased surface area which allowed an even higher glucose uptake leading to a larger placenta with a higher weight (53). Due to the increase in glucose transport, the fetus is exposed to hyperglycemia leading to higher fat storage which in turn leads to increased levels of insulin that induces faster growth, resulting in macrosomia(48,52,53). Clinical guidelines recommend women with GDM to be induced in week 38-39 due to higher incidence of macrosmia, in an attempt to reduce complications such as need for C-section (54). Our study showed a higher nenonatal birth weight, as well as higher placental weight in the GDM placental group compared to normal placentas, however this finding wasn't statistically significant. The explanation may be the fact that most women (84%) found to have GDM by the 1 step approach in gestational week 24-28 were hospitalized. During their hospital stay they were under a strict protocol including healthier diet and regular glucose measurements. Hospitalization in addition

with the regular measurements is thought to contribute to their increased awairness and compliance towards a better glucose control by a strict diet. Another very important contributing factor is that labour was often induced before completion of 40 weeks of gestation affceting the final weight of both the newborn and the placenta. Older age of women has been shown to increase the risk for development of GDM in pregnancy (14, 58). In our study the mean age of women with diagnosed GDM was estimated to 33 years while the control group was somewhat younger with an avarge age of 30 years. The difference of three years was shown not to be statistically significant altought studies have shown that older maternal age is an independent risk factor that increases after 25 years of age (55). Male neonates were more frequently born to GDM mothers as described by Dagelic et al but without statistical significance (52).

Our studies major limitation is the small sample size, including only 24 placentas. The samples were collected from the same department and hospital which isn't representative of the whole population with GDM. Another limitation is the fact that women were screened at week 24-28 which doesn't exclude the possibility of preexisting so called overt diabetes that was undiagnosed up until then. Our study was the first one to describe immunohistochemical expression of GLUT-9 in GDM placentas separately for DC, EVTB, VTB and VSM, thus offering new insights into GDM and GLUT-9. Further studies are necessary in order to understand the impact of GLUT transporter in placentas from GDM, as well as other type of diabetes in pregnancy.

6. CONCLUSION

In conclusion this study shed a light on Immunohistochemical expression of GLUT-9 in GDM placentas. In order to minimize the risks of hyperglycemia and the effects on pregnancy outcomes an early intervention by strict diet control along with frequent glucose measurements are of outmost importance. In women with GDM that are not on insulin therapy, GLUT-9 transporter seems to be expressed in the same quantities as in healthy pregnancies. Since insulin therapy has been shown to increase expression of GLUT transporters, namely GLUT-9 further investigations should be done in this group of women in order to conclude its importance in GDM.

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8. SUMMARY

Objectives: To investigate GLUT-9 immunohistochemical expression in decidual cells (DC), extravillous trophoblast (EVTB), villous trophoblast (VTB) and vasculosyntitial membranes (VSM) in placentas from pregnancies complicated with gestational diabetes mellitus (GDM) and to compare the results with placnetas from normal pregnancies. Secondary outcome was to determine neonatal birth weight and placental weight in both study groups and to compare the results.

Materials and methods: The study included 13 placentas from pregnancies complicated with GDM, and 11 placentas from normal pregnancies served as a control group. GLUT-9 immunohistochemical expression was noted separately for DC, VTB, EVTB and VSM in placentas from in studied placentas.

Results: There was no statistically significant difference in the GLUT-9 immunohistochemical expression among studied groups in all of the studied placental compartents. Neonatal birth weight and placental weight were higher in GDM group compared to control group, however the finding wasnt statistically significant.

Conclusions: The results of our study indicate there is no difference in GLUT-9 immunohistochemial expression between studied groups, which supports the thesis that GLUT-9 isn't a key player in placental glucose uptake when there's no need for insulin treatment of GDM.

9. CROATIAN SUMMARY

Naslov: IZRAŽAJ GLUT-9 U LJUDSKIM POSTELJICAMA KOD TRUDNICA SA GESTACIJSKIM DIABETESOM MELLITUSOM REGULIRANOG DIJETOM

Ciljevi: Istražiti imunohistokemijski izražaj GLUT-9 u decidualnim stanicama (DC), ekstraviloznom trofoblastu (EVTB), vilozusnom trofoblastu (VTB) i vaskulosincijskim membranama (VSM) u postiljicama iz trudnoća kompliciranih s gestacijskim dijabetes melitusom (GDM) i usporediti rezultate s placentama iz normalnih trudnoća. Sekundarni ishod bio je određivanje novorođenačke porođajne mase i mase posteljice u obje ispitivane skupine i usporediti rezultate.

Materijali i metode: Istraživanjem je ispitano 13 posteljica iz trudnoća kompliciranih GDM, a 11 posteljica iz normalnih trudnoća služilo je kao kontrolna skupina. Imunohistokemijski izražaj GLUT-9 zabilježena je odvojeno za DC, VTB, EVTB i VSM u posteljicama od svih ispitivanih posteljica.

Rezultati: Nije bilo statistički značajne razlike u imunohistokemijskom izražaju GLUT-9 među ispitivanim skupinama u svim ispitivanim dijelovima posteljice. Neonatalna porođajna težina i težina posteljice bile su veće u GDM skupini u usporedbi s kontrolnom skupinom, međutim rezultat nije bio statistički značajan.

Zaključci: Rezultati naše studije su pokazali da nema razlike u imunohistokemijskom izražaju GLUT-9 između ispitivanih skupina, što podupire tezu da GLUT-9 nije ključni igrač u unosu glukoze u posteljici kada nema potrebe za inzulinskim liječenjem GDM-a.

10. CURRICULUM VITAE

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