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Analyzing serum tryptophan metabolites in patients with gestational diabetes mellitus

Muammer Özdemir[1*], Sedat Abusoglu[1], Suleyman Baldane[2], Cem Onur Kıraç[2], Ali Unlu[1], Duygu Eryavuz Onmaz[1], Murat Çelik[2], Gulsum Abusoglu[3]

1. Biochemistry, Selcuk University Medical Faculty, Turkey
2. Endocrinology, Selcuk University Medical Faculty, Turkey
3. Medical Laboratory Techniques, Selcuk University Vocational School of Health, Turkey

ABSTRACT

Introduction: Although pregnancy is a physiological condition, the secretion of diabetogenic hormones such as growth hormone, corticotropin-releasing hormone, placental lactogen hormone, prolactin and progesterone from the placenta could lead to insulin resistance (IR). In Metabolic Syndrome, obesity and Type1&2 diabetes, a shift in the kynurenine pathway (KP) towards IDO activation is observed. The activation of the IDO leads to the activation of the Aryl hydrocarbon receptor (AhR) and Interleukin-6 (IL-6) also, which may also induce some effects like insulin resistance, β-cell disfunction and increased gluconeogenesis. We hypothesized that the overactivation of IDO and some KP enzymes would be observed in GDM patients, in a similar manner to metabolic syndrome, prediabetes, and diabetes patients.

Methods: 50 patients and 50 controls, who applied to the Endocrinology outpatient clinic of Selcuk University Faculty of Medicine were included. Serum triptophan metabolite levels were measured with liquid chromatography tandem mass spectrometry.

Results: Tryptophan and KYNA values was found to be lower in the patient group diagnosed with GDM ($p<0.001$ and $p<0.001$, respectively). The levels of KYN, 3-OH AA, 3-OH-KYN and KTR were significantly higher in the patient group compared to the control group ($p=0.008$, $p<0.001$, $p=0.05$ and $p<0.001$, respectively).

Conclusions: Understanding the changes that occur in this pathway in GDM patients may provide insight into the development of the disease. Also these tests could be used as supplementary tests in gestational diabetes, which could assist in diagnosis and patient follow-up.

Keywords: OGTT, gestational diabetes mellitus, tryptophan-kynurenine pathway

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INTRODUCTION

The tryptophan-kynurenine pathway [(Trp-KYN or as Kynurenine Pathway (KP)] is the major pathway for the conversion of tryptophan in humans. Tryptophan is degraded by 4 pathways, but the kynurenine pathway (KP) accounts for approximately 95% of dietary tryptophan (Trp) utilization, of which 90% is due to hepatic KP. In the activation of the immune system, small changes in the peripheral tissues play an active role. [1]

The kynurenine pathway (KP) is regulated by the hepatic tryptophan 2,3-dioxygenase (TDO), as well as the extrahepatic indolamine 2,3-dioxygenases (IDO) 1 and 2 [1]. These enzymes catalyze the conversion of tryptophan to N-Formylkynurenine, which is the first step and rate-limiting step in the pathway. N-formyl kynurenine is converted to kynurenine (Kyn) by the formamidase enzyme. The kynurenine/tryptophan (Kyn/Trp) ratio is a biomarker for the activation of indoleamine 2,3-dioxygenase (IDO) [2].

Kynurenine (Kyn) is then metabolized mainly by two pathways: hydroxylation to 3-hydroxykynurenine (3-OH KYN) by kynurenine hydroxylase (KMO) or to kynurenic acid (KYNA) by kynurenine aminotransferase (KAT A). Either Xanthurenic acid (XA) is synthesized by the Kynurenine aminotransferase [KAT (B)] enzyme, or 3-hydroxy anthranilic acid (3-OH AA) is synthesized by kynureninase (B), from 3-OH KYN. Kynureninase (A) can also hydrolyze kynurenine to anthranilic acid (AA). 3-OH AA is oxidized and unstable metabolite acroleyl amino fumarate is formed. Then, with a nonenzymatic reaction, Quinolinic acid (QA) is formed and also NAD+ or, the nonenzymatic formation of picolinic acid is observed. However, this conversation depends on the level of the substrate

* Correspondence to: Muammer Özdemir, Biochemistry, Selcuk University Medical Faculty, Turkey. E-mail: drmuammerozdemir@gmail.com

saturation of enzyme 2-aminomuconic acid semialdehyde dehydrogenase (ACMSD) [1].

Kynurenine metabolites have a wide range of biological effects, including both pro-inflammatory and anti-inflammatory actions. For example, kynurenic acid (KYNA) is regarded as a neuroprotective and anti-inflammatory metabolite; while antagonizing the methyl D-aspartate receptors (NMDAR), Quinolinic acid (QA) is an NMDAR agonist, neurotoxic and inflammatory metabolite; 3-Hydroxy-kynurenine (3-OH KYN) and 3-Hydroxy anthranilic acid (3-OH AA) are reported as oxidant, neurotoxic and pro-inflammatory metabolites [3, 4].

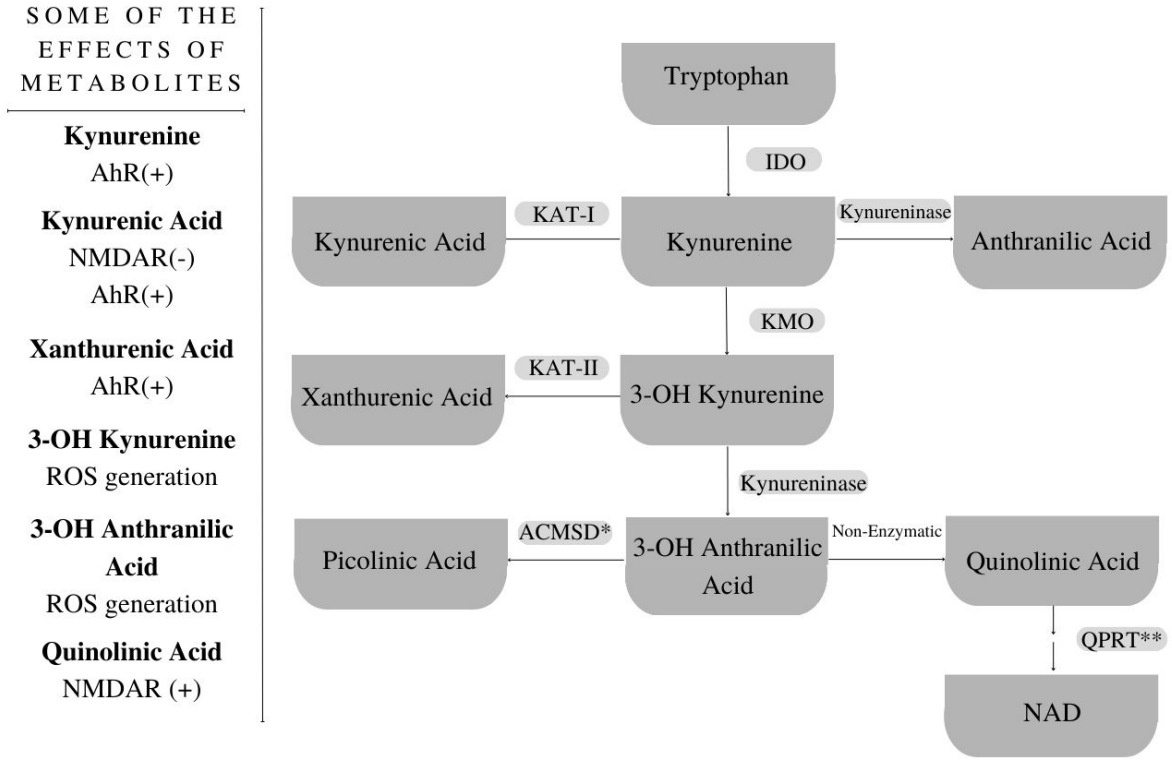
Activation of the KP can also lead to the activation of the Aryl hydrocarbon receptor (AhR), which can be activated by KYN, KYNA and XA [4, 5]. Activation of AhR has been shown to induce several effects, including inflammation, mitochondrial dysfunction, insulin resistance, β-cell dysfunction and increased gluconeogenesis [6] (Figure 1).

Gestational Diabetes Mellitus (GDM) is associated with many side effects and diseases that can lead to miscarriage and fetal death. With treatment, the risk of disease in the mother and the newborn (such as preeclampsia and macrosomia) can be reduced.

According to the traditional definition, a person who has an abnormal glucose measurement at any time during pregnancy is called GDM [7]. Nowadays, however, gestational diabetes is understood to be the type of diabetes that occurs in the second or third trimester in a person who has not had diabetes before. However, this definition does not include individuals with previously undiagnosed type 2 diabetes. The definition of "overt diabetes" is used for these patients. Also, genomic studies have already shown that there is already a strong connection between GDM and Type 2 DM [8] (Figure 2).

The Trp-KYN plays a key role in pregnancy as an immunosuppressive mechanism. The mother's immune system changes during pregnancy, first to protect the fetus from tissue rejection and later to facilitate delivery.

IDO (Indoleamine 2,3-dioxygenase) is involved in processes that protect the fetus from the attack of the maternal immune system by creating a microimmunotolerant environment, by preventing T-cell activation. IDO is produced by blastocysts from the sixth day of gestation, and this production continues throughout pregnancy by syncytiotrophoblasts, extravillous cytotrophoblasts, villous stroma and macrophages in fetal membranes. This synthesis is triggered by the chorionic gonadotropin hormone. Any problem in this mechanism can lead to impaired brain development [9], preeclampsia and even miscarriage [10].



*2-amino 3-carboxymuconate semialdehyde decarboxylase
**Quinolinic acid phosphoribosyl transferase

Fig. 1. Kynurenine Pathway and some of the effects of metabolites (IDO=indoleamine 2,3-dioxygenase, KMO =Kynurenine Monooxygenase, KAT=kynurenine aminotransferase)

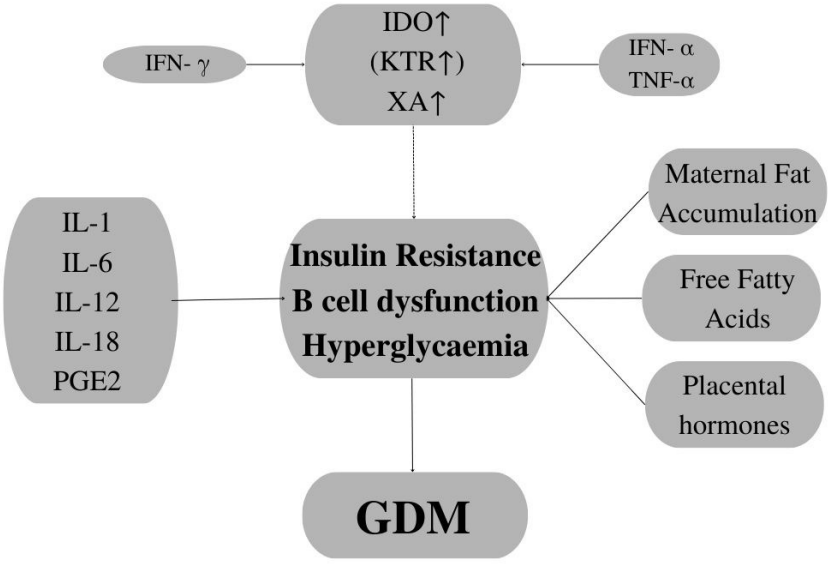


Fig. 2. The pathogenesis of gestational diabetes mellitus (GDM)

Although pregnancy is a physiological condition, the secretion of diabetogenic hormones such as growth hormone, corticotropin-releasing hormone, placental lactogen hormone, prolactin and progesterone from the placenta could lead to insulin resistance (IR). Also, maternal fat accumulation, excess lipolysis, and the release of free fatty acids (FFAs), inflammatory mediators, and adipokines are responsible for IR [3]. In the third trimester, this metabolic state becomes dominant. These changes generally disappear after giving birth [4].

In Metabolic Syndrome, obesity and Type 1&2 diabetes, a shift in the kynurenine pathway (KP) towards IDO activation is observed [2, 11]. IDO is induced by Interferon-gamma (IFN- γ), Interferon alpha (IFN-α), and Tumor necrosis factor alpha (TNF- α) in most cells, which are proinflammatory cytokines. IL-1, IL-12, IL-18 and PGE2 are also proinflammatory molecules and induce IFN- γ [12].

The Trp-KYN pathway has been shown to be impaired in many diseases such as cancer, infections, neurological and psychiatric disorders, kidney diseases, cardiovascular disease, autoimmune and endocrine disorders, as well as metabolic syndrome, prediabetes and diabetes [13]. The deterioration of this pathway is attracting the attention of various researchers, and studies continue to develop a treatment via this pathway. [13, 14]. Unfortunately, there are not many studies evaluating the KP in GDM.

In diabetes, there is a shift in the kynurenine pathway KP towards activation of IDO, as evidenced by elevated serum levels of kynurenine [15]. The involvement of TRP–KYN metabolism in the pathogenesis of diabetes might be further supported by the anti-diabetic effect of berberine [16], known to inhibit KYN formation from TRP [17].

We hypothesized that the overactivation of IDO and some KP enzymes would be observed in GDM patients, in a similar manner to metabolic syndrome, prediabetes, and diabetic patients. Understanding the changes that occur in this pathway in GDM patients may provide insight into the development of the disease. Also these tests could be used as supplementary tests in gestational diabetes, which could assist in diagnosis and patient follow-up. Thus, our aim was to measure serum metabolite levels of the kynurenine pathway in GDM.

METHODS

Patients

The study included 50 patients diagnosed with GDM, [(according to Turkish Endocrinology and Metabolism Association (TEMD) diagnosis, treatment, and follow-up guidelines of diabetes mellitus and its complications)] and admitted to the Selcuk University Faculty of Medicine Endocrinology outpatient clinics, having a single pregnancy, over 18 years of age and below 45 years of age. Also, 50 healthy pregnant women with normal physical examinations and normal routine test results were involved in our study as the control group [18].

Any patients with previous Type 1 or Type 2 DM, acute or chronic illness (Emergency room or outpatient clinic admissions such as hyperemesis, cold, enteritis, etc., or previously known diseases such as cancer, infections, neurological and psychiatric disorders, kidney diseases, cardiovascular disease, autoimmune disorders like rheumatoid arthritis, sjögren, Behcet etc.), acute or chronic infections, using diabetogenic drugs, smoking, preeclampsia, and eclampsia, being under 18 years of age or over 45 years of age were the causes of exclusion from our study. Measurements were in accordance with

TEMD guidelines and were performed in 26th week of gestation, in both groups. [18]

This study was approved by the Ethics Committee of Selcuk University Faculty of Medicine (Number: 2019/84, Date: 08.04.2019).

Analysis

The counts of lymphocytes, neutrophils, monocytes, and eosinophils were determined using an automated blood cell counter (Beckman Coulter LH 780, California, USA).

The concentrations of all glucose parameters were determined using the hexokinase method. HbA1c and CRP were measured using the turbidimetric method. Insulin and ferritin were measured using the immunoassay method. AST, ALT, cholesterol, LDL, HDL, and triglyceride were measured using the Cobas 6000 analyzer (Roche Diagnostics). The homeostatic model assessment of insulin resistance (HOMA-IR) is calculated by the formula "fasting glucose (mg/dL)*fasting insulin (uIU/L)/405".

Tryptophan analysis

Serum tryptophan levels were quantitated via LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometry) ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) as described by a previously reported method [19] with slight modifications. 300 µL serum, calibrators and controls were mixed with 100 µL internal standard (donepezil) and 1000 µL reducing reagent acetonitrile (including %1 formic acid) and vortexed for 30 seconds. After vortexing and centrifugation at 12000 rpm for 10 minutes, 40 µL supernatant was used for LC MS/MS analysis.

Statistical analysis

Statistical analysis was performed using the SPSS statistical software program version 27. Descriptive statistics for the data were presented as mean ± standard deviation (width: minimum-maximum), median, number (n), and percentile (%). Independent sample t-tests and Mann-Whitney U tests were used to compare the demographic and clinical characteristics and laboratory findings of the study participants according to the study groups. In addition, other laboratory parameters of the patients were investigated with Spearman's rho correlation coefficient. For statistical tests, the significance level was $p < 0.05$.

RESULTS

The mean age of the patient group was lower than that of the healthy control group, with a mean age of 26 (18-39) years versus 28 (23-38) years, respectively (p=0.063). Demographic and laboratory data are presented in Table 1.

KYN levels [288 ng/mL (140-2460) in patient group and 228 ng/mL (56-790) for controls, p=0.008]; 3-OH AA levels [7.59 ng/mL (3.94-28.4) in patient group and 3.45 ng/mL (0.56-11.6) for controls, p<0.001] and 3-OH-KYN levels [2.29 ng/mL (0.61-6.68) in patient group and 2.08 ng/mL (0.51-5.90) for controls, p=0.05] were higher in patient group, also with the kynurenine-tryptophan ratio (KTR) [0.037 (0.02-0.13) in patient group and 0.02 (0.005-0.10) for controls, p<0.001].

TRP levels [7350 ng/mL (3360-18990) in patient group and 10972,50 ng/mL (5175-20800) for controls, p=0.001) and KYNA levels [3.08 ng/mL (0.89-8.03) in patient group and 4.67 ng/mL (1.54-22.30) for controls, p<0.001] were significantly lower in patient group. All of the KP metabolites level comparisons are presented in Table 2.

Moreover, neutrophil count was correlated with tryptophan, (R-value -0.320, p=0.004) KTR, (R value 0.393, p<0.001) and 3-OH KYN (R value 0.349, p=0.002) levels. Basophil, HbA1c and CRP values were significantly correlated with 3-OHAA levels (R-value 0.290, p=0,009; R-value 0.252, p=0,046 and R-value 0.352, p=0,035 respectively). The eosinophil count was slightly correlated with KYNA (R-value 0.226, p=0,045) (Table 3).

DISCUSSION

It is known today that metabolic syndrome, obesity, insulin resistance and GDM could cause diabetes, but the underlying biochemical mechanisms mediating the effects of stress and inflammation are not clearly known.

It has been shown in many studies that there is a predisposing link between insulin resistance, obesity, and Trp-KYN and NAD+ pathways. Obesity is a major risk factor for insulin resistance [20]. Also inflammation is showed to be in adipose tissue by IDO1 expression and other inflammatory factors like TNF-a and INF-g.

In the Shanghai diabetes study, high serum TRP levels were observed in patients who developed type 2 DM. These patients were followed for 10 years. Also, the patients with high TRP levels had insulin resistance and high insulin secretion. In another study, there was no significant difference in tryptophan levels between the Type 2 DM group and the control group [21]. Although high TRP levels are suggested as a predictive marker, it is in contrast to the pregnant patient group in our study, in which we found low tryptophan levels, because of the possible activation of IDO enzyme.

In the knowledge of existing literature, pro-inflammatory molecules (e.g. IFN-γ) [22] and/or stress hormones (e.g. cortisol) activate the induced TRP-KYN pathway en-

Table 1. Laboratory parameters in GDM and healthy subjects

	GDM (n = 50)	Controls (n = 50)	p*
Age, years	26 (18-39)	28 (23-38)	0.063
HbA1c, %	5.3 (4.8-6.1)	4.85 (3.3-5.9)	0.006
FPG, mg/dL	92 (66-179)	84 (72-101)	0.094
PPG, mg/dL	103 (63-119)	-	-
Insulin, uIU/mL	10.1 (6.2-19.6)	8.3 (1,6-13,1)	<0,001
HOMA-IR**	2,34 (1.16-7.07)	1.60 (0.35-3,27)	<0,001
OGTT 50-1, mg/dL	147 (71-188)	-	-
OGTT 100-0, mg/dL	93.5 (69-111)	-	-
OGTT 100-1, mg/dL	174 (50-246)	-	-
OGTT 100-2, mg/dL	154 (68-217)	-	-
OGTT 100-3, mg/dL	120.5 (66-144)	-	-
AST, U/L	17 (11-30)	17 (10-67)	0.644
ALT, U/L	13 (7-25)	12 (7-49.27)	0.462
CRP (mg/L)	5.18 (0-10)	3.23 (0.103-8)	0.143
Cholesterol mg/dL	210 (143-287)	179 (129-278)	0.084
LDL cholesterol, mg/dL	112.20 (68-178)	106 (60-160)	0.202
HDL cholesterol, mg/dL	54 (38-73)	52.5 (34-85)	0.541
Triglyceride, mg/dL	163 (84-263)	92 (39-470)	0.010
Ferritin, mg/dL	11.19 (2-34)	15.63 (1-118)	0.332
NE, $*10^3$/ul	6.90 (4.19-14.80)	3.90 (1.72-8.3)	<0.001
LY, $*10^3$/ul	1.9 (1.02-4.28)	2 (1.1-5.14)	0.914
BA, $*10^3$/ul	0.036 (0-0.781)	0.02 (0-0.116)	0.010
EO, $*10^3$/ul	0.075 (0-2.38)	0.1 (0-1.3)	0.081

FPG: Fasting Plasma Glucose, HOMA-IR: Homeostatic Model Assessment Insulin Resistance, PPG: Post-Prandial Glucose, OGTT: Oral Glucose Tolerance Test, AST: Aspartate Transaminase, ALT: Alanine Transaminase, CRP: C-Reactive Protein, LDL: Low density lipoprotein, HDL: High density lipoprotein. All values are presented as mean, min-max and p value. *Bold values indicate p < 0.05, **Calculated with formula "FPG*insulin/405"

Table 2. Kynurenine metabolites in GDM and healthy subjects

	Patient Group (n =50)	Control Group(n =50)	P value*
Tryptophan, ng/mL	7350 (3360-18990)	10972.50 (5175-20800)	<0.001
Kynurenine, ng/mL	288 (140-2460)	228 (56-790)	0.008
KTR	0.037 (0.022-0.129)	0.019 (0.005-0.097)	<0.001
KYNA, ng/mL	3.08 (0.89-8.03)	4.67 (1.54-22.30)	<0.001
3OH-AA, ng/mL	7.59 (3.94-28.4)	3.45 (0.56-11.6)	<0.001
3OH-KYN, ng/mL	2.29 (0.61-6.68)	2.08 (0.51-5.90)	0.05

KTR: Kynurenine/Tryptophan Ratio, KYNA: Kynurenic Acid, 3 OH-AA: 3-Hydroxy-Anthranilic Acid, 3-OH-KYN: 3-Hydroxy-Kynurenine, *Bold values indicate p < 0.05

zymes IDO, TDO (Tryptophan 2,3 dioxygenase), and KMO (Kynurenine Monooxygenase) in the development of IR, Type 2 DM and possibly Type 1 DM (There is a shift in the pathway toward inflammation) [3, 20]. This shift in the pathway combined with PLP (Pyridoxal phosphate) deficiency causes the formation of metabolites that affect insulin activity, like Xanthurenic Acid (XA) [23,24]. Adipocytes synthesize macrophage chemotactic protein-1 (MCP-1) and vascular and intracellular adhesion molecules (VCAM and ICAM), which enhance the interest of lymphocytes and monocytes. Subsequently, these cells engage in interactions with adipocytes, giving rise to macrophage recruitment, synthesis of inflammatory cytokines, and compromised adipocyte functionality, thereby inducing IR [3]. Reduced IDO1 expression has been observed in pancreatic beta cells in type 1 DM, and cell damage due to possible loss of immune tolerance is thought to be a major factor in disease development (17).

In experimental studies trying to correct IDO1 activity in non-obese diabetic rats, it has been shown that the development of tolerance to autoantigen in dendritic cells can be recovered *in vivo* [25,26]. Also, the lifespan of pancreatic islet cells can be extended [27] and normoglycemia can be achieved [28].

In the study of Piras et al., the levels of TRP were found to be significantly higher and the levels of KYN and 3-OH KYN were found to be significantly lower in the urine of patients with GDM. The developing fetus has a high demand for tryptophan during pregnancy, as TRP plays a number of important roles in fetal development, including protein synthesis, brain development, and overall physiology, as reported in this study. Also, the authors hypothetised that the activation of KP may be due to the low grade inflammatory state in obese as well as in GDM patients [29]. Contrary to our findings, it is possible that TRP is excreted in the urine during the

Table 3. Descriptive statistics and correlation coefficients of kynurenine pathway and laboratory parameters

Variable	M	SD	1	2	3	4	5	6	7	8	9	10	11
1. Age, years	28.97	4.93											
2. Trp, ng/mL	9816.39	4128.03	0.096										
3. KYN, mg/mL	300.79	247.40	0.04	0.09									
4. KTR	0.03	0.02	-0.016	-0.610**	0.669**								
5. KYNA, ng/mL	4.76	3.54	0.009	0.326**	0.16	-0.097							
6. 3OH-AA, ng/mL	6.47	4.23	-0.073	-0.235*	0.234*	0.333**	-0.312**						
7. 3OH-KYN, ng/mL	2.43	1.31	0.116	-0.165	0.068	0.216*	0.017	-0.007					
8. HbA1c, %	3.52	2.54	0.18	-0.211	0.178	0.234	-0.171	0.252*	0.085				
9. FPG, mg/dL	88.58	14.51	0.028	-0.008	0.086	0.054	-0.093	0.074	0.011	0.415**			
10. Insulin, uIU/mL	9.41	3.67	-0.009	-0.122	0.132	0.196	-0.057	0.179	0.115	0.363**	0.675**		
11. HOMAIR	2.15	1.14	-0.005	-0.106	0.123	0.171	-0.093	0.17	0.092	0,391**	0,796**	0,978**	
12. PPG, mg/dL	97.91	18.14	0.071	0.055	-0.109	-0.374	-0.173	-0.497	0.319	-0.232	0.351	-0.005	0.132
13. OGTT 50-1, mg/dL	140.21	36.54	-0.004	0.037	-0.249	-0.171	0.236	-0.03	0.114	0.299	0.229	0.569*	0.468
14. OGTT 100-1, mg/dL	170.43	41.92	0.325	0.038	0.246	0.2	-0.107	-0.417	0.124	0.295	0.657**	0.477*	0.537*
15. OGTT 100-2, mg/dL	152.90	30.49	0.262	-0.103	0.06	0.231	-0.116	-0.222	0.018	0.479	0.282	0.266	0.326
16. OGTT 100-3, mg/dL	111.13	25.47	0.113	-0.539*	-0.134	0.361	-0.16	-0.255	0.009	0.327	0.131	-0.096	-0.046
17. AST, U/L	18.75	8.43	0.057	-0.001	-0.213	-0.169	0	-0.016	0.02	-0.01	-0.001	-0.132	-0.086
18. ALT, U/L	15.07	8.74	0.134	-0.027	0	0.039	0.023	0.021	0.154	0.144	0.279*	0.118	0.165
19. CRP, mg/dL	14.91	61.85	-0.053	-0.07	-0.113	-0.064	-0.247	0.352*	0.069	0.481**	0.16	0.111	0.137
20. Cholesterol, mg/dL	191.18	43.27	-0.049	-0.131	0.175	0.185	-0.15	0.117	0.072	-0.004	-0.113	0.084	0.057
21. LDL, mg/dL	110.79	31.33	0.091	-0.159	0.179	0.229	-0.19	0.085	0.101	0.052	-0.017	0.173	0.15
22. HDL, mg/dL	53.72	12.42	-0.173	0.061	0.023	-0.005	-0.162	0.208	-0.223	-0.088	-0.125	-0.17	-0.152
23. Triglyceride, mg/dL	136.05	80.60	0.099	-0.135	0.088	0.073	0.011	0.185	0.31	0.162	0.011	0.1	0.094
24. Ferritin, mg/dL	20.06	20.84	0.089	-0.303*	0.128	0.253	0.207	-0.009	0.104	0.234	0.039	-0.059	-0.012
25. NE, *10^3/ul	5.52	2.51	-0.191	-0.320**	0.163	0.393**	-0.171	0.349**	0.274*	0.294*	0.092	0.240*	0.222
26. LY, *10^3/ul	2.10	0.71	-0.057	0.097	0.051	-0.073	0.034	0.091	-0.105	-0.165	-0.045	-0.002	-0.009
27. BA, *10^3/ul	0.05	0.10	-0.018	-0.091	0.059	0.091	-0.015	0.290**	0.075	0.113	0.063	0.098	0.098
28. EO, *10^3/ul	0.17	0.32	-0.043	0.147	-0.125	-0.207	0.226*	0.016	-0.015	-0.028	0.119	0.05	0.068

** $p < 0.01$ (2-tailed); * $p < 0.05$; N=100

12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
-0.6																
0.643	0.238															
-0.679	0.343	0.3														
0.5	0.144	-0.091	0.415													
-0.325	-0.031	-0.358	0.078	0.326												
-0.199	0.173	0.224	0.21	0.085	0.628**											
-0.5	0.36	-0.203	0.754	0.359	0.054	0.089										
0.4	-0.36	0.31	0.061	0.156	-0.124	-0.287	0.035									
0.2	-0.27	0.261	0.042	0.071	-0.173	-0.299	0.024	0.949**								
0.2	-0.721	0.213	-0.287	0.205	-0.222	-0.208	0.059	0.534**	0.506**							
0.4	-0.09	0.285	0.055	0.024	0.303	0.061	0.022	0.444**	0.27	-0.242						
0.321	0.486	-0.429	0.286	1.000**	0.169	0.214	0.033	0.011	0.029	0.115	-0.11					
0.061	-0.074	0.201	0.066	0.361	-0.272*	-0.085	0.422*	0.264	0.195	0.216	0.268	0.001				
0.113	-0.142	-0.167	-0.524*	-0.464	-0.292*	-0.06	0.146	0.067	-0.015	0.053	0.021	0.227	-0.072			
-0.009	0.097	-0.109	-0.26	0.224	-0.086	0.001	0.251	-0.094	-0.14	-0.072	0.105	-0.21	0.199	0.326**		
0.116	-0.155	-0.092	-0.301	-0.234	-0.014	0.027	0.339	-0.242	-0.299	-0.229	0.025	0.205	-0.211	0.256*	0.037	

transition to full-state diabetes, as suggested by Yu et al. [30].

The KTR has been suggested as a potential risk marker for the development of type 2 diabetes mellitus (T2DM) in patients with coronary artery disease. An increase in the rate of KTR in the urine increases the incidence of hypertension and Type 2 DM [31]. It was shown in a 7-year study that the measurement of KTR in the urine could be a risk marker for developing diabetes [32].

KTR was measured higher in the patient group compared to the control group in our study. (Median values were 0.037 (0.022-0.129) in the patient group and 0.019 (0.005-0.097) for controls, p<0.001) KTR was also found to be high in obesity, which could advance to Type 2 DM [33]. Obesity is also characterized by low-level inflammation in the white adipose tissue caused by many inflammatory mediators, including KYN [34,35].

In our study, the TRP levels were significantly lower in the patient group (p=0.001), while the KYN levels were significantly higher in the patient group (p=0.008). Consequently, the KTR was found to be higher in the patient group (p<0.001). These findings are consistent with the majority of previous studies mentioned above (Figure 3).

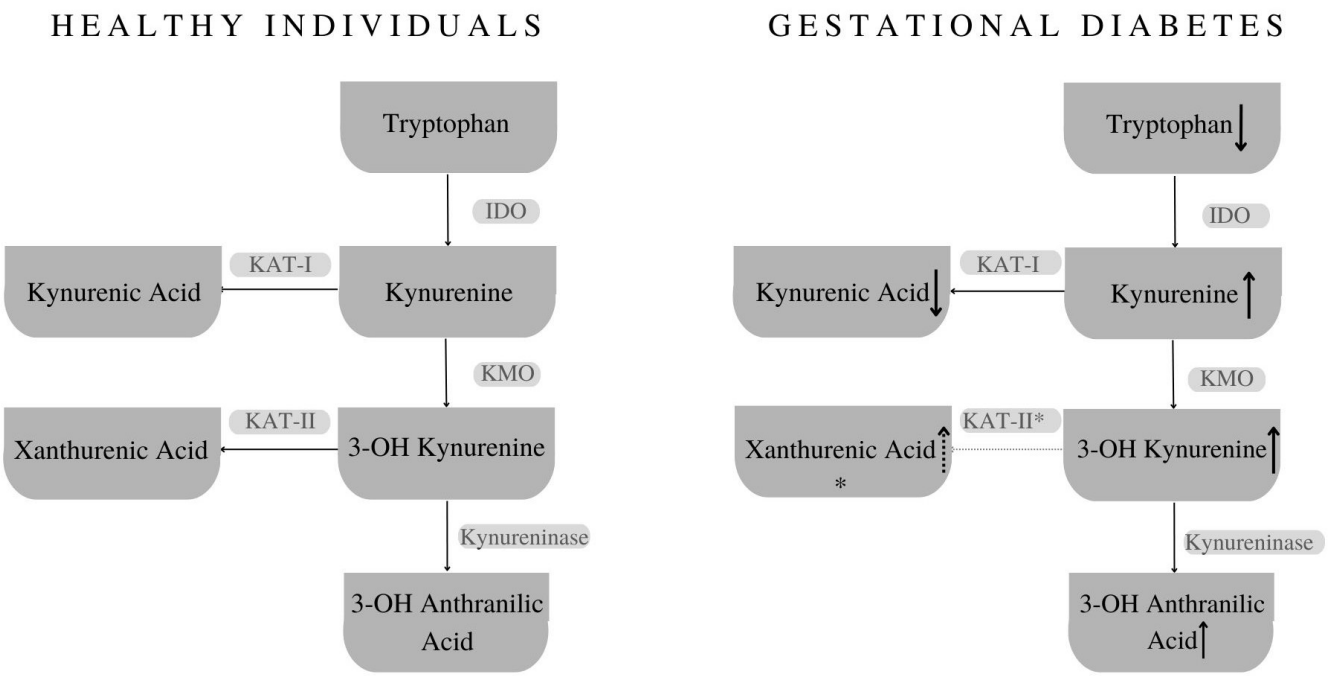
Favennec et al. reported that increased KMO expression in adipose tissue is correlated with HbA1c levels in their study [3]. This is consistent with our findings, as we also observed that 3-OH KYN levels are slightly correlated with HbA1c (R-value 0.252, p=0,046). Peripheral inhibition of KMO has been suggested to prevent obesity and diabetes [2, 36]. In our study, 3-OH KYN, which is a precursor of XA and a marker of KMO expression and activity, was found to be significantly higher in our patient group (p=0.005)

KYNA and QA act as an antagonist on the α7 nicotinic acetylcholine receptor (α7nAChR) and ionotropic glutamate receptors [37]. Also, among the ionotropic glutamate receptors, KYNA preferentially inhibits the N-methyl-D-aspartate receptor (NMDAR) [38]. KYNA was found to be increased compared to the control group in a study performed on Type 2 DM patients [21]. Contrary to this finding, we found a decrease in KYNA in our analyses [(3.08 ng/mL (0.89-8.03) in patient group and 4.67 ng/mL (1.54-22.30) for controls, p<0.001)].

The incidence of preeclampsia and eclampsia is increased in women with GDM [39, 40]. Elevated levels of KYNA have been found higher in a previous study, performed in preeclampsia patients [41]. However, contrary to our expectations, we found that KYNA levels were lower in the patient group. This finding is both distinct and significant, suggesting that a different mechanism may underlie the development of preeclampsia and eclampsia in pregnant women with GDM.

The kynurenine pathway metabolites XA and KYNA have been shown to inhibit proinsulin synthesis in isolated rat pancreatic islet cells [42].

In Gestational Diabetes a study conducted on 14 pregnant women, it was shown that oral pyridoxine may benefit blood insulin, OGTT, or urinary xanthurenic acid excretion levels [43]. In another study performed on 13 pregnant women, it was shown that oral pyridoxine did not provide a significant change in blood insulin, OGTT,



* Xanthurenic acid levels have been known as high in prior studies [29].

Fig. 3. Comparison of the metabolites in GDM and Healthy Subjects

or urinary xanthurenic acid excretion levels, in contrast to the previous study [44].

In previous studies, 3-OH AA levels have been found to be lower in type 2 DM compared to the control group [21]. In our study, we found high levels of 3-OH AA ($p<0.001$). We believe that this finding represents a significant contribution to our understanding of the relationship between 3-OH AA levels and type 2 DM.

Although XA levels were not measured in our study, the observed increase in 3-OH KYN levels suggests a shift toward activation of the KAT-B enzyme within the kynurenine pathway.

Quinolinic acid is a metabolite that has neurotoxic properties. Its neurotoxic effects are augmented by an increase in glutamate release within the synaptic gap, a decrease in glutamate reabsorption into astrocytes, production of reactive oxygen species, a reduction in endogenous antioxidant molecules and lipid peroxidation [45].

The strengths of our study are that KYN, KTR, 3-OH KYN and 3-OH AA levels were found significantly higher, and KYNA levels were found significantly lower in GDM patients compared to the control group. Understanding the changes that occur in this pathway in GDM patients may provide insight into the development of the disease. They could also be used in the treatment process, in measuring the effectiveness and adequacy of the treatment.

Limitations

Unfortunately there were limitations to our study. The sample size of this study was limited due to the stringent selection criteria and challenges in identifying participants who fulfilled all the requirements. Therefore, the study did not include any metric parameters like height, weight, BMI, waistline and total adipose tissue etc.

CONCLUSIONS

This study provided new insights into the relationship of GDM with Tryptophan metabolites and the kynurenine pathway. While GDM is a multifactorial disease influenced by factors such as waist circumference, weight, total adipose tissue, and genetics, its diagnosis is based on laboratory results from patient samples. Further research involving large and diverse populations is necessary to account for the influence of environmental factors, genetics, and lifestyle on patient outcomes across different ethnic groups. Our study of the KP in the development of GDM is a novel finding that opens up a number of questions.

ABBREVIATIONS

α7nAChR: α7 nicotinic acetylcholine receptor

3-OH-AA: 3-Hydroxy-anthranilic acid

3-OH-KYN: 3-Hydroxy-kynurenine

ACMSD: 2-aminomuconic acid semialdehyde dehydrogenase

AA: Anthranilic acid

AhR: Aryl hydrocarbon receptor

AST: Aspartate transaminase

ALT: Alanine transaminase

BA: Bazophil number

BAP: Scientific Research Projects Department of Selcuk University,

CRP: C-reactive protein

EO: Eosinophil number

FFA: Free Fatty Acids

FPG: Fasting plasma glucose

GDM: Gestational diabetes mellitus

HDL: High-density lipoprotein

HOMA-IR: Homeostatic model assessment of insulin resistance

IDO: Indoleamine 2,3-dioxygenase

IFN: Interferon

IL: Interleukin

IR: Insulin Resistance

KAT: Kynurenine Aminotransferase

KMO: Kynurenine Monooxygenase

KP: Kynurenine pathway

KYNA: Kynurenic acid

KYN: Kynurenine

KTR: Kynurenine/Tryptophan ratio

LDL: Low-density lipoprotein

LY: Lymphocyte number

MCP-1: Macrophage Chemotactic Protein-1

NE: Neutrophil number

NMDAR: N methyl D-aspartate receptor

NFκB: Nuclear Factor kappa-B

OGTT: Oral glucose tolerance test

PPG: Post Prandial Glucose

QA: Quinolinic acid

T2DM: Type 2 Diabetes Mellitus

Trp: Tryptophan

Trp-KYN: Tryptophan-Kynurenine pathway

TDO: Tryptophan 2,3-dioxygenase

TNF- α: Tumor necrosis factor

XA: Xanthurenic acid

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AUTHORS' CONTRIBUTIONS

Conceptualization: OM; methodology: OM, AS, BS; software: BS, CM; validation, OM, AS, BS, AG; formal analysis: OM, EOD; investigation: OM, AS, BS, KOC, UA, EOD; data curation, OM, BS, KOC, EOD, CM, AG; writing-original draft preparation: OM; writing-review and editing: OM, AS, BS, KOC, UA, EOD, AG; visualization: MO, AS; supervision: OM.

CONFLICT OF INTEREST

None to declare.

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