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High Incidence of *Demodex folliculorum* in Women Treated for Gestational Diabetes Mellitus Persists after Treatment

Mustafa Kaplanoglu¹, Mehmet Bulbul², Tuncay Celik³

¹ Istinye University Faculty of Medicine, Istanbul, Turkey.
 ² Department of Obstetrics and Gynecology, Karabuk University Faculty of Medicine, Karabuk, Turkey.
 ³ Department of Parasitology, Adiyaman University Faculty of Medicine, Adiyaman, Turkey.

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Corresponding author:

Mustafa Kaplanoglu, MD, Assoc Prof.

ORCID: 0000-0002-4658-1336

Istinye University School of Medicine,

Istanbul, TURKEY

mustafakaplanoglu@gmail.com

ABSTRACT

Demodex so is the most common ectoparasite in humans. Its intensity increases especially in diabetes mellitus and chronic diseases. In our study, demodex density was evaluated after blood glucose regulation in patients with gestational diabetes mellitus (GDM). In our prospective study, 50 g glucose challenge test and 100 g glucose tolerance test were performed stepwise at 24-28 weeks of gestation. Patients with gestational diabetes mellitus were followed up and treated. A total of 82 women, 23 with gestational diabetes mellitus, 59 healthy control pregnant women, whose blood glucose was regulated within the specified period were included. Skin demodex densities were evaluated after blood glucose regulation of the patients. There was no difference between the two groups in terms of obstetric characteristics, sociodemographic data, age, height, weight, miscarriage and gestational week. Demodex infestation was found in 10 (43.5%) of the pregnant women in the GDM group and in 12 (20.3%) of the pregnant women in the control group, and this difference was statistically significant. The incidence of demodex infestation in gestational diabetes is higher than in healthy pregnant women. This rate continues even when blood glucose is under control.

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INTRODUCTION

Demodex sp. is one of the most common parasites of the human body. Although it has more than 140 members, the most common forms in humans are Demodex folliculorum and D. brevis. These are saprophytic parasites that live in hair follicles and sebaceous glands (1). It was first described by Henle in 1841. The two most common groups, D. folliculorum, were described in 1841 and D. Brevis in 1963 (2). Although D. folliculorum can be found all over the body, it is mostly the sebaceous glands on the cheeks, eyelids, nose, forehead, and chin. It has an average length of 0.3-0.4 mm, an average lifespan of 14-16 days, and usually moves at night. Although they are very common, they usually do not cause any obvious clinical pathology. However, local parasite density should be <5 parasites/cm2 for significant disease. Demodex infestation is associated with chronic diseases such as cancer, diabetes mellitus and chronic kidney failure, and immunodeficiency states such as HIV infection (3,4).

Gestational diabetes mellitus (GDM) is a glucose intolerance condition that is diagnosed for the first time during pregnancy

and can be seen in 18% of pregnancies. The main pathogenetic mechanism in GDM is the inability to meet the increased insulin requirement by pancreatic β cells due to decreased insulin sensitivity during pregnancy. Increased glucose and insulin amounts and insulin resistance in the tissue increase susceptibility to various infections in GDM patients (5). Accordingly, the decrease in the migration of neutrophils and monocytes to the area of inflammation suppresses the immune response. When the physiological immunosuppressive environment of pregnancy is added to this, the susceptibility to infection may become more pronounced (6). Diet and insulin are the main steps in the management of GDM patients. It is controversial which of the relative immune suppression due to pregnancy and immune change due to diabetes is dominant in the possible etiology of demodex infestation in GDM patients.

In our present study, the presence of demodex infestation between GDM pregnant women with blood glucose regulation and healthy control group was evaluated comparatively. In this way, the persistence of the effect of GDM on demodex infestation was evaluated.

METHODS

The presented study was planned as a prospective controlled study. In the power analysis, it was planned to include 90 volunteers with a confidence interval of 90% and an error of 8.5%. The study approval was obtained from the Ethics Committee of Adiyaman University Faculty of Medicine (No: 2020/9-35).

All patients with GDM who were followed up in Adıyaman University Faculty of Medicine, Department of Obstetrics and Gynecology between 01.11.2020-30.04.2021 and who met the study criteria were included in this study.

Patients demographic characteristics, obstetric anamnesis and BMI of the patients were recorded in the study. During the pregnancy follow-ups, a 50 g 1-hour oral glucose test was applied to the patients who were called for screening between 24-28 weeks. Pregnant women with normal results were included in the control group. In the 1st hour evaluation, 100 g glucose tolerance test was applied for 3 hours to the patients whose blood glucose was above 140 mg/dl. Higher 2 out of 4 cut-off values were diagnosed as GDM. Cut-off values were determined according to Carpenter and Coustan criteria. (6) These patients were started on diet and/or insulin therapy. The patients were followed up according to weekly fasting, 1st and 2nd hour blood sugars. Values below 90 mg/dl, 140 mg/dl and 120 mg/dl were considered normal, respectively. Patients with diet-regulated blood sugar were included in our study.

Exclusion criteria were as follows: History of endocrine immunosuppression pathology, cancer, any status. dermatological disease (such as Systemic Lupus facial seborrheic dermatitis, rosacea, Erythomatosus, blepharitis), chronic disease (such as kidney failure, liver failure), and smoking-alcohol use.

Sample materials were taken from the face, cheek, nasolabial and chin regions of the individuals participating in the study by Standard Superficial Skin Biopsy (SYDB) method. In this method, first a 1 cm2 area was drawn on a clean slide, then 1 drop of cyanoacrylate was dropped on the other side of the slide, in the middle of this area, and the surface to be sampled was pressed and slowly lifted after approximately 1 minute. Afterwards, the names and surnames of the participants, the codes representing the region where the sample was taken, and the date were written on the samples, put in the slide transport box and taken to Adıyaman University Faculty of Medicine, Department of Medical Parasitology for examination within 1 hour. D. folliculorum mites density was examined using light microscopy (Olympus CH20; Olympus Optical, Tokyo, Japan) at ×40 and ×100 magnification. The identification of >5 mites/cm2 of skin was defined as D. folliculorum mite infestation.

Statistical analysis

Data analysis was performed using SPSS version 23 (IBM, Chicago, IL, USA) package program. In the analysis of variables with continuous value, Mann Whitney U test for those with non-normal distribution. Categorical data were compared with chi-square tests. Continuous values were expressed as mean \pm standard deviation (SD) and categorical data as n (%). P values less than 0.05 were considered for statistical significance.

RESULTS

During the study, 59 patients in the control group and 23 patients in the GDM group agreed to take samples for *Demodex* (Table 1).

Table 1. Clinical and demographic results of the groups.

	Control (n=59) [n(%)]	GDM(n=23) [n(%)]	р
Demodex	12 (20.3)	10 (43.5)	0.034
Previous operation history	23 (39.0)	8 (34.8)	0.725
	Control (n=59) [mean±SD]	GDM(n=23) [mean±SD]	
Age (year)	30.9±6.4	33.7±6.8	0.090
Gravida	2.9 ± 1.7	3.9 ± 2.1	0.048
Parity	1.6±1.3	2.3±1.9	0.043
Abortion	$0.4 {\pm} 0.8$	0.6 ± 0.8	0.319
Height (cm)	161.6±5.2	159.9±3.5	0.153
Weight (kg)	80.5±13.0	82.0±13.4	0.634
Abdominal circumference (cm)	105.3±13.6	112.4±16.2	0.048
Gestational age (week)	30.8±4.6	32.5±4.7	0.151
HbA1c (%)	5.4 ± 0.8	6.1±0.7	0.002
Haemoglobin (g/dL)	12.2±1.3	12.6±0.9	0.242
Thrombocyte (10 ³ /μL)	218.3±52.6	221.4±44.2	0.166
WBC $(10^3/\mu L)$	10.7±3.1	9.4 ± 2.3	0.046
fT3 (pg/mL)	2.9 ± 0.5	2.8 ± 0.5	0.575
fT4 (ng/dL)	0.62 ± 0.19	0.68 ± 0.34	0.292
TSH (µIU/mL)	1.76±1.05	2.16±1.27	0.156

SD: Standard deviation, GDM: Gestational diabetes mellitus, WBC: White blood cell, TSH: Thyroid stimulating hormone.

There was no difference between the two groups in terms of sociodemographic data, age, height, weight, abortion and gestational week (p>0.05). Gravity, parity and abdominal circumference were found to be statistically significantly higher in the GDM group (p<0.05).

When the laboratory results were compared, the mean HbA1c values were higher in the GDM group as expected (p=0.002). There was no difference between the groups in terms of mean hemoglobin, thrombocyte, free T3, free T4 and TSH values (p>0.05). In addition, mean leukocyte values were lower in the GDM group than in the control group (10.7±3.1 vs 9.4±2.3, p: 0.046). HbA1c was different between groups as predicted. (5.4±0.8 vs 6.1±0.7, p: 0.002)

Demodex infestation was found in 10 (43.5%) of the pregnant women in the GDM group and in 12 (20.3%) of the pregnant women in the control group, and this difference was found to be statistically significant (p=0.034).

DISCUSSION

The *Demodex* ectoparasite is one of the most common parasites in humans. Its detection even in the neonatal period suggests that the demodex ectoparasite is a member of the normal skin flora. On the other hand, *D. foliculorum* is present in 23-100% of people below the infestation limit of 5 mites/cm2 (7-9). The density of demodexin in the skin varies in relation to many pathological conditions. Immunodeficiency conditions such as cancer or chronic diseases (CR renal failure, diabetes mellitus...) are the most common causes. The association of GDM with demodex infestation has been shown previously (10).

Demodex mites are associated with pathologies such as blepharitis, and some pathological mechanisms have been suggested in the etiology. Direct epithelial cell damage, reactive hyperplasia and hyperkeratinization, and mechanical obstruction of the meibomian gland flow pathway are the most common causes. These areas provide a suitable environment for bacterial settlement (especially staphylococci). Here, both demodex and bacterial exotoxin cause tissue damage through irritation and hypersensitivity reaction (11). In inflammatory lesions, changes in the immune system and hormonal balance (such as polycystic ovary syndrome, puberty), low chemotactic activity of neutrophils, decrease in mastocyte function, weak leukocyte-endothelial cell interactions and leukocyte reduction, low oxidant compound production, decreased lymph node retention capacity and decreased tumor necrosis factor alpha, interleukins, and prostaglandins can alter the intensity of demodexin through the release of cytokines (11,12).

Pregnancy theoretically causes changes in the immune system and this is considered as selective immunosuppression. In a limited number of studies, it is stated that pregnancy is not a risk factor for demodex mite infestation (13). However, there is more definite evidence for diabetes mellitus (14). Therefore, it can be thought that the increased demodex infestation in GDM is not due to pregnancy but to the direct effect of DM.

The study we present differs from previously published studies involving the relationship between diabetes mellitus or GDM

and demodex. All these studies have proven the relationship between diabetes and demodex. Kurt et al. found that demodex infestation was significantly different between the healthy control group and GDM patients (24.2% vs. 3.3%, respectively).

Similarly, Akdeniz et al. (15) showed that the *Demodex* density of diabetic patients was significantly different from the healthy control group. In the study of Clifford and Fulk (5), which included 256 elderly diabetic patients, it was determined that the risk of demodex infestation increased with age, especially in eyelash examination. A similar increase was detected in *Staphylococcus aureus* (16). This result showed that *Demodex* density changes with age and colonization of other bacteria. Although all these studies show the relationship between diabetes and demodex, there is not enough information about the continuation of infestation after treatment.

In our present study, the presence of demodex in the first month after blood glucose regulation of GDM patients was evaluated and a continued increase in demodex density was found (20.3% vs 43.5%). In addition to being a chronic pathology, DM can cause serious damage especially to the endothelium. Repair of these damages is often not possible. Demodex mites are localized in areas such as the pilosebaceous unit. Increased density in these regions and endothelial damaging pathologies such as DM may create an escape site from the immune system in advanced infestation. Therefore, it seems possible to detect high demodex skin density for a long time after the disappearance of pathologies such as DM. The high posttreatment demodex density in our present study can be explained in this way. The most important weakness of our study is the relatively low number of patients. Higher patient numbers will reveal both the pregnancy demodex relationship and the chronic diseases and demodex density more comprehensively.

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