Original Article

The Role of Insulin Level on the Biofilm-Forming Capacity in Diabetes-Related Urinary Tract Infection

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is more prone to get infections and the most common infection is urinary tract infection (UTI), most of the causative agents are related to biofilms, biofilm-forming capacity affected by host factors such as glucose and others. **Aims:** The objective of this research was to see how insulin affects the biofilm-forming capacity that most common pathogens associated with diabetic patients in different isolates. **Materials and Methods:** The objective was investigated by comparing the amounts of serum insulin in UTI patients to those without UTI whether the patients with T2DM or nondiabetic. The study was conducted on 40 T2DM patients divided into 20 patients with UTI and 20 without UTI, and 40 nondiabetic control subjects 20 with UTI and 20 patients without UTI. Serum insulin levels were detected by using enzyme-linked immunosorbent assay kit. **Results:** The mean concentration of serum insulin was a highly significant increase in T2DM in comparison to the nondiabetic control group. *Pseudomonas auroginosa* was the strongest biofilm producer isolate. **Conclusion:** In conclusion, insulin's direct effect was elevated the capability of biofilm formation. This contributes to a better knowledge of the causes of frequent bacterial infections in diabetics.

Keywords: Biofilm, type 2 diabetes mellitus, urinary tract infection

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by high blood glucose levels as a result of insulin impairment and/or decreased insulin sensitivity.[1] Because of the hyperglycemic environment, which changes immune function such as neutrophil dysfunction, phagocytosis, and chemotaxis, diabetic individuals are more susceptible to infections.[2] The most prevalent infection in diabetic individuals is urinary tract infection (UTI), particularly cystitis.[3,4] The glycosuria promotes the growth of a wide range of bacteria strains.[5] The fundamental issue with UTIs is their recurrence and duration, which is caused by the existence of a biofilm-associated pathogen.[6] Biofilms are microbial communities of surface-attached cells encased in an extracellular polymeric matrix that they manufacture themselves. Biofilm formation is thought to be a determinant of long-term infections.[7] Biofilm production and composition are influenced by a variety of environmental conditions and substances, including glucose, which promotes biofilm formation.[8] Pathogens which cause such 1infections, is1exposed to numerous host factors including hormone insulin.[5] Insulin is defined as an endocrine hormone that binds to receptors on target cells' plasma membranes.[9]

The aim of this study was to see how insulin affected the expression of virulence factors in bacteria that are commonly linked with diabetic patients and cause biofilm formation.

Materials and Methods

This study was conducted on 200 patients attending at Al-Suwayrah hospital between November 2020 and March 2021. One hundred and twenty patients from them were T2DM (the diagnosis of T2DM was made based on the recommended criteria by the American Diabetes Association)[10] age range within 30–51 years. Eighty samples were collected to be comparable to DM patients in respect

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to age and gender and selected among patients who were nondiabetic, nonhypertensive, -no other-endocrine disorders or metabolic kidney diseases and subjected into two groups: with UTI and without UTI.

Type 2 diabetes mellitus patients

Fifty-three from the 120 T2DM patients were with UTI as confirmed by bacterial growth. The other patients were T2DM without UTI. Forty-six of them were with biofilm-forming capacity (bacterial isolates) and for the purpose of comparison, only 20 T2DM with UTI were included. The other patients were 20 T2DM without UTI were selected to be matched in respect to age and gender with nondiabetic groups.

Control subjects

For the purpose of comparisons, 80 Iraqi nondiabetic patients comparable to DM patients in respect to age (30–51 years) and gender (24 females and 16 males), were included in the study, only 24 of them were with biofilm-forming capacity (bacterial isolates). To be comparable to other groups the participants were subjected into two groups: 20 subjects with UTI and 20 without UTI.

Urine samples collection

Urine examination and bacterial cultivation were performed on each sample. The subject considered infected with UTI after bacterial isolation. In accordance with a clean-catch procedure, the participants were asked to provide a morning, midstream urine sample of about 30 ml. The sample collected was divided into two parts, the first part for immediate measurement for the general physical, chemical, and microscopic examination. Urine cultures were performed on the second part of the urine specimen.

Blood sampling and collection

From each patient, 3 ml of blood were withdrawn after 8 h-fasting. The blood specimen was separated into two parts-: The first one was used for the estimation of fasting plasma glucose (FPG) level. The second half was distributed in a plain tube and allowed to clot at room temperature (22°C) for about an hour before being centrifuged at 3000 rpm for 10 min and blood collected to determine serum insulin levels. It was stored in Eppendorf tubes at (−20°C) until it was needed.

Enzyme linked immmunesorbent assay test

Serum insulin level estimation was quantitatively determined in diabetics and nondiabetic controls with UTI and without UTI using sandwich enzyme linked immmunesorbent assay test using the commercially available kit, Human serum insulin (BT LAB, China). The absorbances were read in a microplate reader (Human, Germany) and were calculated by interpolation from a standard curve that was performed using a curve fitting equation.

Results

The FPG revealed that the mean increased significantly of the T2DM group in comparison to controls (255.97 ± 108.64 vs. 96.90 ± 12.29 mg/dL). The T2DM patients showed an increased mean of serum insulin in comparison with controls (21.31 ± 9.81 vs. 4.96 ± 2.42) and this increase was highly significant [Table 1].

Biofilm-forming capacity among bacterial isolates

The microtiter plate method was used in the quantitative biofilm assay. The results showed that out of 113 (57%) bacterial isolates, 70 (61.40%) isolates were biofilm producers which showed blue colors appearance. While 43 (38.59%) isolates colonies indicating no biofilm production.

As shown in Table 2, biofilm-forming capacity of *Pseudomonas aeruginosa* in the T2DM group was strong producer with mean absorbance of (0.580 nm). *Staphylococcus aureus* was also strong biofilm producer with mean absorbance of (0.575 nm), in contrast *Klebsiella pneumoniae* and *Escherichia coli* were moderate in biofilm-forming capacity and with mean absorbance of (0.417 nm *vs.* 0.362 nm) respectively.

Table 3 shows a biofilm-forming capacity in nondiabetic group and demonstrated that *Pseudomonas aeruginosa* means of absorbance (0.576 nm) and *S. aureus* mean absorbance (0.573 nm) both bacteria were strong producers.

Table 1: Means level of fasting plasms glucose and serum insulin in type 2 diabetes mellitus and control groups

Parameter	Mean±SE		*P*
	Patients (*n*=40)	Controls (*n*=40)	
Serum insulin (μIU/ml)	21.31±9.81	4.96±2.42	0.000
Fasting plasma glucose (mg/dL)	255.97±108.64	96.90±12.29	0.000

SE: Standard error

Table 2: Results of biofilm-forming capacity in diabetic patients with urinary tract infection

Bacterial isolates in diabetic patients	Biofilm-forming capacity	Mean OD (nm) OD values at
Pseudomonas aeroginosa	Strong	0.580
Staphylococcus aureus	Strong	0.575
Klebsiella spp.	Moderate	0.417
Escherichia coli	Moderate	0.362

Table 3: Results of biofilm-forming capacity in the nondiabetic group with urinary tract infection

Bacteria isolates in diabetic patients	Biofilm-forming capacity	Mean OD (nm)
Pseudomonas	Strong	0.576
Staphylococcus aureus	Strong	0.573
Klebsiella spp.	Moderate	0.390
Escherichia coli	Moderate	0.327
Staphylococcus saprophyticus	Moderate	0.488

While *Klebsiella* spp., *E. coli* and *Staphylococcus saprophyticus* were moderate producers and the mean OD were (0.390 nm), (0.327 nm), and (0.488 nm), respectively.

Bacterial proliferation with insulin-administration-under—in-vitro conditions

Regarding the bacterial ability to develop and multiply in diverse environmental conditions, bacterial isolates which were isolated from clinical-urine samples revealed after the administration of human insulin at a dose of 2.5 U/ml, at various incubation periods (0, 6, and 12 h). The absorbance was read at 600 nm wavelength nonsignificant difference in proliferation rate with different isolates was observed. Although growth with insulin was more than without [Table 4].

Insulin role on biofilm formation

Results showed that the administration of insulin to the clinical isolates give a stimulatory effect on biofilm formation. The biofilm-forming capacity was dramatically increased after adding hormonal insulin in a dose of 2.5 U/ml in all bacterial isolates from the studied groups as shown in Table 5.

Discussion

T2DM affects individuals who have insulin resistance that defined as a lower biological activity of the insulin hormone in its different metabolic actions for a certain concentration. There is insulin hypersecretion which compensates for the lack of hormonal action.[11] Biofilm-associated pathogens which cause such-infection, is exposed to a variety of host variables, including the hormone insulin.[5] The results of biofilm formation capacity in 113 isolates were 70 (61.9%) isolates. A similar result was reported by Al-Hamadany[8] in Iraq with a percentage of 60% of tested isolates had the ability to produce biofilm. Another study identified a slightly greater percentage of biofilm producers, with 63.3% of isolates being biofilm producers.[12] The fact that biofilm in *S. aureus* and *Pseudomonas* are higher than *E. coli*, *Klebsiella* in both diabetic and nondiabetic groups[13] stated that biofilm ability makes the antibiotics are ineffective in reducing symptomatic infections, and raises the risk of UTIs and bacteriuria (bacteria in the urine) by three to six percent per day. Insulin concentrations of 2.5 U/ml, enhanced the growth rate of bacterial isolates in this study**.** Similar observations have been reported by Plotkin and Viselli[14] who found the rate of growth for *S. aureus* as with *E. coli, Pseudomonas aeroginosa* increased at effective concentrations of insulin and glucose. Insulin has been shown to alter the growth kinetics of Gram-positive and Gram-negative bacteria, implying that the ability to respond to this hormone is widespread in nature. Insulin alone had no influence on *E. coli* production time. With 0.1% glucose, the combination of insulin and glucose altered growth in such a way that the growth rate was enhanced.[14] Studies indicated that *E. coli's* growth kinetics are directly affected by insulin. The presence of glucose or a substrate that can be converted to glucose is required for the effect to occur. Insulin-dependent glucose transport can be seen in insulin-dependent mammalian tissue, where insulin binding to its receptor causes the upregulation of glucose transport protein production on cell membranes.[15]

It has been shown that mammalian hormones such as insulin can affect bacterial growth rate, gene expression, pathogenicity (including biofilm formation), and antibiotic susceptibility.[14,16-18] On the other hand, not only hormones but also nutritional factors such as sugars in the bacterial habitat could also affect some biological processes, such as the expression of virulence genes,[19,20] and alteration of metabolic pathways.[20]

As mammalian cells coordinate by synthesizing hormones to regulate and maintain their own homeostasis, bacteria coordinate community behavior via chemical signaling which are known as quorum sensing (QS) to optimize their resources, defense, survival, virulence, and antibiotic_resistance. Gram-positive and Gram-negative bacteria used auto inducing peptide (AIP) for communication via the QS s. This signaling process not only occurs between bacterial cell to cell but also occurs between bacteria and their hosts.[21,22] It is well known that hormones are one of the host's factors which determine the environmental conditions of a pathogen and insulin is the most common and pervasive of these hormones.[14] The effects of insulin and glucose on the expression of virulence factors were concentration specific. Some of the insulin effects seen could

Table 4: Bacterial proliferation after insulin administration

Group	Bacteria in diabetic	Zero time	Growth without insulin after 6 h	Growth with insulin after 6 h	Growth without insulin after 12 h	Growth with insulin after 12 h	*P*
Diabetic	*Pseudomonas*	0.086	1.570	1.580	1.701	1.720	0.978
Non diabetic control		0.068	1.590	1.600	1.660	1.680	
Diabetic	*Staphylococcus aureus*	0.065	1.53	1.54	1.719	1.727	0.748
Non diabetic control		0.063	1.520	1.530	1.713	1.720	
Diabetic	*Klebsiella*	0.093	1.090	1.178	1.596	1.594	0.819
Control		0.091	1.077	1.156	1.396	1.398	
Diabetic	*Escherichia coli*	0.089	1.369	1.474	1.613	1.593	0.310
Non diabetic control		0.090	0.960	1.032	1.10	1.080	
Non diabetic control	*Staphylococcus saprophyticus*	0.088	0.855	0.515	1.900	1.790	

Table 5: OD of biofilm-forming capacity after insulin addition

Group	Bacteria	OD with insulin	OD without insulin	*P*
Diabetic	*Pseudomonas*	1.300	0.290	0.0001**
Control		1.239	0.270	0.0001**
Diabetic	*Staphylococcus aureus*	1.286	0.271	0.0001**
Control		1.101	0.265	0.0001**
Diabetic	*Klebsiella* spp.	0.780	0.270	0.0308*
Control		0.900	0.240	0.0015**
Diabetic	*Escherichia coli*	0.751	0.214	0.0376*
Control		0.730	0.190	0.0329*
Control	*Staphylococcus saprophyticus*	1.080	0.260	0.0001**

*$P \leq 0.05$, **$P \leq 0.01$

be due to the presence of homoserine lactone (AI-1) or cyclic borate diester (AI-2) autoinducers of QS and altered virulence factor synthesis, either directly or indirectly.[23-25] Insulin combined with glucose enhances epithelial cell adhesion, which is thought to be the first step in the colonization of host mucosal surfaces. It is widely known that *E. coli* cultured in glucose-containing media has a greater propensity to adhere to uroepithelial cells.[26] The results of this investigation show that insulin boosts epithelial cell adhesion by boosting the effect of glucose. A variety of adhesive elements may be involved in this adhesion.,[26,27] which are affected by insulin including fimbriae.

Previous studies showed that Insulin and glucose have been demonstrated to influence *E. coli* behavior, particularly the production of biofilms.[28,29] Recombinant human insulin influences *E. coli* biofilm formation in a substrate and microenvironment-dependent manner, according to studies. Results from this study indicate that insulin in conjunction with glucose promotes biofilm development.[30] Biofilm generation was shown to be higher in diabetes and no diabetic patients, with no statistical correlation between the two groups. Though the uropathogen's biofilm-forming capacity was reported to be greater *in vitro*[6] and showed that the human hormone insulin, served as an autoinducer for the production of biofilms.[2,31] Assume that insulin treatment can aid in the spread of *E. coli* infection in diabetic patients, because it can cause the expression of some virulent factors, which can then act as a signal molecule for QS and biofilm formation, which, in turn, can lead to the need for more medical interventions in diabetic patients.[32]

Individuals with T2DM can excrete insulin (or/and glucose) in their urine, which may explain why these medical disorders have a higher prevalence of severe UTI s.[30] This feature may create a favorable environment for the growth of biofilms.

Conclusion

In conclusion insulin's direct effect was elevated the capability of biofilm formation. This contributes to a better knowledge of the causes of frequent bacterial infections in diabetics.

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Conflicts of interest

There are no conflicts of interest.

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