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Research Article

Correlation of Uropathogenic *Escherichia coli* Harbouring pap Operon with IL-6 and TLR-4 Serum Levels and Other Biomarkers in Diabetic Mellitus Patients

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A B S T R A C T

Introduction: Urinary tract infections (UTIs) are commonly investigated by microbial agents such as *Escherichia coli*, with a significant incidence in diabetic individuals, where chronic hyperglycaemia can exacerbate complications. This study investigates the interplay between UTI pathogens and systemic inflammatory markers in diabetic patients by focusing on the *pap* operon, a gene complex associated with *E. coli* virulence.

Methods: The three study groups comprised 100 diabetic patients with UTI, 100 without UTI, and 100 healthy controls. Some biomarkers (FBS and HbA1c) related to diabetes were measured. Using ELISA, serum TLR-4 and IL-6 levels were quantified, and through PCR, the presence of the *pap* operon was assessed.

Results: Twenty-five samples were found to be positive for *E. coli*. The findings revealed that patients with the *pap* operon had elevated mean TLR-4 levels of 1.72 ng/mL compared to 1.02 ng/mL in the negative cohort, which was a statistically significant difference (p = 0.001). IL-6 levels were similarly higher in the operon-positive group, averaging 86.42 ng/mL versus 68.36 ng/mL in the operon-negative group, again with a significant difference (p = 0.001). However, the variations in FBS and HbA1c levels were not statistically significant, with FBS levels at 120.99 mg/dL for the 'Positive' group and 120.63 mg/dL for the 'Negative' group (p = 0.804), and HbA1c levels at 9.75% and 10.01%, respectively (p = 0.415). Conclusion: These results suggest that while the *pap* operon is associated with an increased inflammatory response, it does not have a direct impact on glucose metabolism indicators in the studied diabetic cohort. The data accentuate the necessity for comprehensive studies to elucidate these relationships further and formulate targeted therapeutic interventions.

Keywords: Cytokines, ELISA, Operon, PCR, Primer

Introduction

Urinary tract infection (UTI) refers to the microbial invasion of any site in the urinary tract, including the kidneys, ureter, bladder and urethra.[1] The most common causative agent of UTI is *E. coli* and other *Enterobacteriaceae*.[2] Hematogenous transmission accounts for 5% of infections and may follow bacteraemia.[3]

Diabetes mellitus (DM) is a group of metabolic diseases characterised by hyperglycaemia, which is a result of insufficient insulin synthesis, action, or both. The pancreas produces the hormone insulin so that body cells can absorb glucose and use it as fuel; without it, hyperglycaemia occurs.[4] Chronic hyperglycaemia may cause a number of complications. Chronic hyperglycaemia, associated with diabetes, can cause long-term damage, dysfunction, and failure of several organs, including the kidneys, eyes, nerves, heart, and blood vessels.[4]

About 80–90% of all UTIs in the community are caused by uropathogenic *E. coli* (UPEC).[5] Four main UPEC phylogroups have been identified (A, B1, B2, and D) based on the presence of genomic Pathogenicity Islands (PAI) and the expression of virulence components such as adhesins, toxins, surface polysaccharides, flagella, and iron-acquisition systems.[6] For UPEC to cause UTI, several of these virulence factors are usually necessary.[7–9] It is important to note that UPEC is not the only pathogen that may cause a UTI; potential offenders include the bacteria *Klebsiella pneumoniae* (about 7%), *Proteus mirabilis* (around 5%), *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Streptococcus bovis*, and the fungus *Candida albicans* (for the remaining percentage).[10]

The *pap* gene cluster comprises 11 genes that encode the primary fimbria rod component (*papA*), adaptor subunit-encoding *papEF*, and the terminal adhesin gene *papG*. The adhesin gene *papG* codes for 4 molecular variations of the *papG* subunit, encompassing classes I–IV, each of which has a unique allele.[11] These four *papG* genetic variations each have unique receptor binding specificities, which are thought to impart variances in host range specificities and/or the ability to produce particular UTI clinical symptoms, despite some contradictory results in other investigations.[12]

Pyelonephritis in adult women and children, acute prostatitis in males, and bacteraemia in a variety of hosts have all been demonstrated to be substantially correlated with *papGII*. The *papGIII* variation, on the other hand, has mostly been reported in cystitis isolates from children, men, and women.[13] The majority of UTI infections seldom include the presence of *papGI*, which is typically seen in very minute quantities in a variety of host populations and clinical conditions. Although the *papGIV* allele has been identified, its distribution and function are still unclear.[14]

Regarding the role the four *papG* polymorphisms play in host specificity, there is still inconsistent evidence in the literature, which is likely due to variations in research methods, population definitions, and geographic contexts.[15]

Therefore, it is still unclear if the 4 *papG* variations vary in terms of related bacterial features, clinical syndromes, or host characteristics in a carefully chosen host group.[16]

Methods

Study Participants

This study involved 300 participants having diabetes and UTI infection (n = 100), diabetes and not suffering from UTI (n = 100) and healthy subjects (controls) with no diabetes and UTI (n = 100). Samples were collected from patients attended the Specialist Center for Deaf Diseases and Diabetes Glands during the period between December 2022 and February 2023. The Ethics Committee of the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, approved the study protocol. The subjects were informed about the voluntary participation and written informed consent was obtained from all before getting information using validated questionnaires. According to exclusion criteria, the patients who suffering from other chronic diseases were excluded. Data from the study were subjected to statistical analysis through the use of SPSS software.

Samples

Blood (3 mL) was collected from each of the participants in the patient and control groups. The blood was subjected to centrifugation for 20 minutes, and then the upper layer of serum was transferred to a tube and used in ELISA. Urine samples collected from each participant included in this study were tested for UTI by culturing on selective media.

Determination of IL-6 and TLR-4 Serum Level by ELISA

The serum levels of human Toll-like receptor (TLR) and human Interleukin 6 (IL-6) were detected using commercial CUSABIO/ China brand ELISA kits. The plate is pre-coated with a human antibody. The sample is added, which binds to antibodies coated on the wells. Then Streptavidin-HRP (horseradish peroxidase) is added, which binds to the biotinylated antibody. After incubation, unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and colour develops in proportion to the amount of human IL-6 and TLR-4. The reaction is terminated by the addition of acidic stop solution and the absorbance is measured at 450 nm.

Distribution of papG Alleles among Uropathogenic *Escherichia coli*

For PCR amplification of the *pap* genes, the mix was conducted using 12.5 Master mix Promega (Promega

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GoTaq™ Green MasterMix) with 1 µL of forward primers, 1 µL of reverse primers, and 3 µL of genomic DNA. Finally, the reaction mix was completed to 25 µL with nuclease-free water. The sequence of the forward primer and the reverse primer of each *pap* gene that was targeted in this study is listed in Table 1. The programme initiated the reaction with a denaturation step at 95 °C for 3 minutes and 30 seconds. This was followed by 40 cycles, each consisting of a denaturation phase at 95 °C for 40 seconds, an annealing phase at 60 °C for 40 seconds, and an extension phase at 72 °C for 40 seconds. Concluding the cycles, a final extension was performed at 72 °C for 5 minutes.

Results

PCR resulted in the successful amplification of the target DNA sequence. The presence of a single band, when compared to a DNA ladder, corresponds to the expected size of 336 337 base pairs (bp) as shown in Figure 1. No other extraneous bands were observed, suggesting that the PCR was specific for the intended target without any non-specific amplification. The absence of multiple or smeared bands indicates that there was no amplification of off-target sequences or primer-dimer formations, respectively. In summary, the PCR protocol effectively amplified the desired DNA fragment of 336 bp in length.

From the 100 patients' samples collected in this study, 25 samples (25%) tested positive for *E. coli*, indicating a significant presence of this bacterial strain within the study cohort. Furthermore, of these *E. coli* positive samples, 16 (64%) were found to carry the *papA* gene, as summarised in Figure 2.

The data presented delineates a comparative analysis of various physiological markers in relation to the presence or absence of the *pap* operon. Notably, there's a pronounced elevation in TLR-4 mean values in *pap* operon positive samples (1.72) ng/mL as compared to their negative counterparts (1.02), ng/mL a difference which is statistically significant with a p value of 0.001. Similarly, the IL-6 levels are markedly increased in the *pap* operon positive group (86.42) ng/mL compared to the negative group (68.36 ng/mL), and this difference is validated by a p value of 0.001. On the other hand, the disparities in fasting blood sugar (FBS), and HbA1c between the two groups are not as pronounced, and their respective p values suggest that these differences might not be statistically significant.

Figure 3 illustrates the mean TLR-4 serum levels in the two distinct groups. Individuals in the 'Positive' group exhibited a higher mean TLR-4 serum level (1.72) ng/mL compared to the 'Negative' group (1.02). ng/mL The difference between the groups was statistically significant (p = 0.001), indicating a strong association between the group classification and TLR-4 serum levels.

Figure 4 shows a comparison between the mean serum levels of IL-6 between the two groups. The 'Positive' group exhibited significantly higher IL-6 serum levels (86.42) ng/mL compared to the 'Negative' group (68.36) ng/mL , as shown by the blue and orange bars, respectively. The difference between groups was statistically significant, with a p value of 0.001, indicating a strong association between the group classification and IL-6 serum levels.

Figure 5 illustrates the mean FBS levels in the two different cohorts. The mean FBS level in the 'Positive' group was 120.99 mg/dL, compared to 120.63 mg/dL in the 'Negative' group. The difference in mean FBS levels between the groups was not statistically significant (p = 0.804), implying that the group classification does not correlate with changes in FBS levels within the populations studied.

Figure 6 shows the mean HbA1c levels for the two study groups. The 'Positive' group had a mean HbA1c level of 9.75%, while the 'Negative' group had a slightly higher mean level of 10.01%. The observed difference in HbA1c levels between the groups was not statistically significant, with a p value of 0.415, indicating no substantial evidence to suggest a difference in glucose control between the two groups over the period assessed.

Table 1.Primer Sequences Used in This Study

Primer Names	Sequence 5'-3'
papA-For	ATGGCAGTGGTGTTTTGGTG[1]
papA-Rev	CGTCCCACCATACGTGCTCTTC[1]
papE/F-For	GCAACAGCAACGCTGGTTGCATCAT[1]
papE/F-Rev	AGAGAGAGCCACTCTTATACGGACA[1]
papGII-For	GGGATGAGCGGGCCTTTGAT[1]
papGII-Rev	CGGGCCCCCAAGTAACTCG[1]

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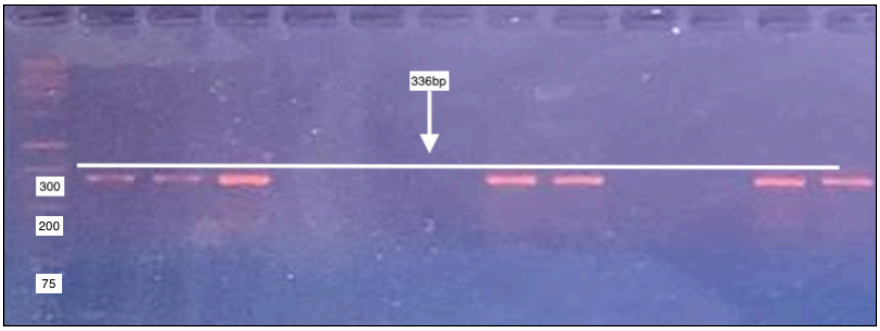

Figure 1.PCR Products that Represent an Approximately 336 bp Segment of the *papA* Gene

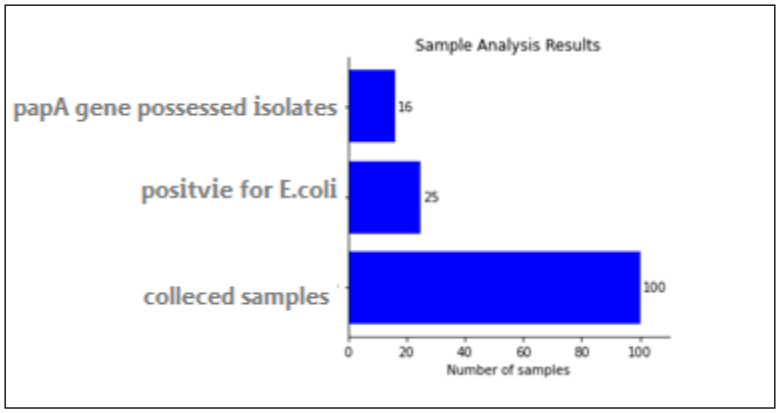

Figure 2.Summarisation Figure 2. Summarisation of Positive *E. coli* collected Samples and Isolates that Possessed the *papA* Gene

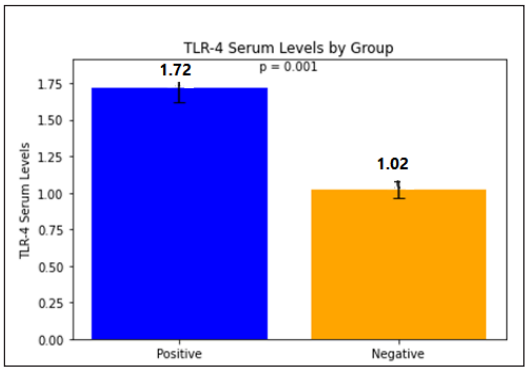

Figure 3.Comparison of TLR-4 Based on the Presence or Absence of *pap* Operon

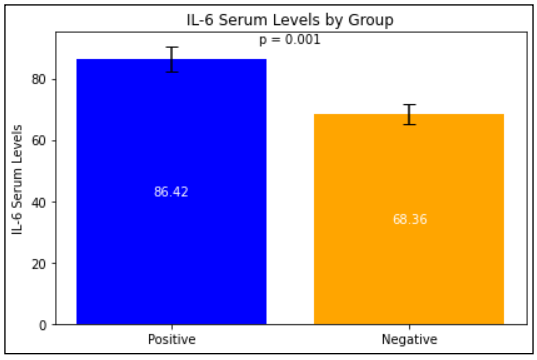

Figure 4.Comparison of IL-6 Based on the Presence or Absence of *pap* Operon

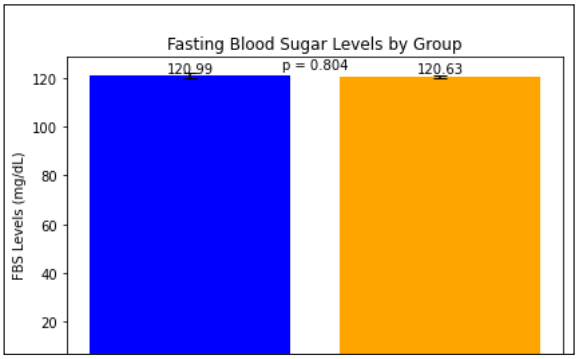

Figure 5.Comparison of FBS Based on the Presence or Absence of *pap* Operon

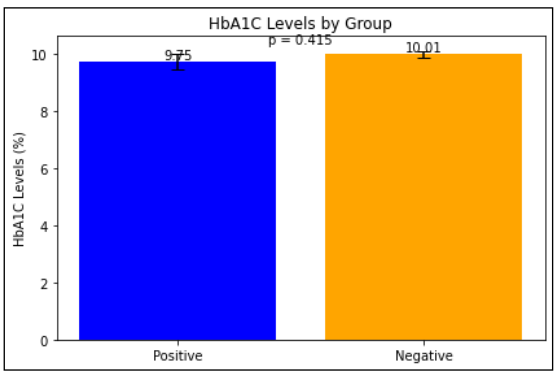

Figure 6.Comparison of HbA1c Based on the Presence or Absence of *pap* Operon

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Discussion

The findings elucidate distinct variations in the levels of a number of physiological indicators depending on the presence or absence of the *pap* operon. The obvious difference in TLR-4 and IL-6 levels between the two groups is one of the most startling findings.

The well-established function of TLR-4 in identifying bacterial lipopolysaccharides may explain the increase in TLR-4 values in *pap* operon-positive samples (LPS). The *pap* operon, which is essential for bacterial adherence and colonisation, may make it easier for TLR-4 to recognise bacterial cells, resulting in a higher inflammatory response.[17] However, it is important to recognise that if this increased inflammatory response is not properly controlled, it may result in tissue damage, putting people at risk for problems. The outcomes of research by Frendéus et al., which also discovered higher TLR-4 levels in *pap* operon-positive samples, support these findings.[18]

The higher IL-6 levels in the *pap* operon-positive group may also point to an ongoing infection or inflammatory activity. High IL-6 levels have previously been linked to bacterial infections, indicating that the body's natural defences are designed to get rid of the invaders.[19] The *pap* operon's role in bacterial pathogenicity and the associated host inflammatory response may be the direct cause of the rise in IL-6. These findings are consistent with the studies of Koley et al., which also found elevated IL-6 levels in *pap* operon-positive samples.[20]

It is difficult to tell the two groups apart based on the data of MIR, FBS, and HbA1c, and their statistical insignificance shows that their roles in relation to the *pap* operon are not clear-cut. Despite the fact that FBS and HbA1c are principally linked to glucose metabolism and long-term blood glucose regulation, respectively, their minor differences in regard to the *pap* operon suggest a less direct connection. Secondary effects on glucose metabolism may result from persistent infections or long-lasting inflammatory conditions that are characterised by the presence of the *pap* operon. However, in the absence of hard data from this dataset, it is still a speculative direction that demands further research.

These results are in line with those of research by Wilson and Biggel et al., who likewise found no significant variations in FBS and HbA1c levels between the two groups.[21,13] However, a study by Rhee presented contradictory data by reporting distinct differences in these parameters based on the presence of the *pap* operon.[22]

In order to fully understand these connections, it is necessary to perform longitudinal studies including a variety of groups and use cutting-edge methods like genomics and proteomics to reveal the complex mechanisms at work. The development of targeted treatment strategies to lessen the negative effects of bacterial genes like the *pap* operon and hence improve patient outcomes depends on the successful translation of these results into clinical practice.

Conclusion

The research emphasises a connection between uropathogenic *Escherichia coli* carrying the *pap* operon and increased levels of TLR-4 and IL-6 in the blood of diabetic patients. This suggests that there is an intensified response. This suggests that there is an intensified response to infection with *E. coli* carrying *the pap operon, which* could lead to stimulation of TLR-4 and IL-6. However, no direct impact was observed on markers of glucose metabolism. These findings highlight the interaction between factors that cause disease and the immune responses of diabetic individuals. They also emphasise the importance of research to develop interventions that can reduce the negative effects of such infections and improve patient outcomes.

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Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: None

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