Molecular detection of *vicR, vicK* and *16S rRNA* genes of *Streptococcus mutans* Isolated from Dental Caries of Iraqi's Patient

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Abstract

Background : This study was carried out to detect the distribution of *Streptococcus mutans* with dental caries in Iraqi's patient samples. Out of 100 patient's samples only (22) with age (3-17) years old were found to be effected with *Streptococcus mutans*. DNA was extracted amplified by Gradient PCR and then sequenced. vicR was showed highly conserved with no mutation while *vicK* showed that these were silent mutation and four isolates have missense mutation from eight isolate.

Material & Method : In this study, 22 from clinical *S. mutans* strains were isolated from caries- children to sequence the *vicR, vicK* and *16s rRNA* genes. Genomic DNA was extracted from S. mutans strains and amplified using Gradient PCR. The PCR products were purified and then sequenced.

Result : The molecular study include *vicR*, *vicK* and *16S rRNA,* genes by Gradient PCR. The result of sequencing appear that no mutation in *vicR* genes, although in *16S rRNA* present transion and tranvesion mutation . Finally the sequencing of *vicK* gene appear silent mutation and four isolates have missense mutation from eight

Conclusion the result of molecular study of *vicR* gene were appear no mutation in all isolates while *16S rRNA* although have transition and transvertion mutation, the Sequence of *vicK* gene that note more of mutation was silent mutation but some of them was missense mutation.

Keywords: *Dental caries, molecular, Streptococcus mutans.*

Introduction

Dental caries is a major common and common public health oral illness which hinders the attainment and safeguard of oral health in variation age groups [(1)] . Dental caries is one of most important prevalent infectious disease of the human can be leads wastage tooth structure, tthere after changes in oral functions [(2)] . Other define of dental caries is a bacterial disease of the dental hard tissue and happen in certain localized sites in the dentitions. In the iorder of frequency sites to attack "pits" and "fissures", especially on the surfaces of the teeth, proximal contact of the surface, and "labial", "buccal" and "lingual" surface of the dentition neighboring to the gingivae [(3)] . So consider a complex disease caused by a physiological imbalance, between fluid and mineral dental biofilm [(4)] . *Streptococcus mutans* is the plurality cariogenic pathogens in tooth decay. The highly acidogenic, industrialize short-chain acids which

the soften hard tissues of dental. Three isozymes of glucosyltransferases metabolize and catalyze; sucrose to industrialize insoluble extracellular polysaccharides, which increase attachment. The most isolated of significant from tooth caries samples are *S. mutans* [5] In the oral cavity Despite being ubiquitous, *Streptococcus mutans* spread often an indicates caries portability and no good oral hygiene. *Streptococcus mutans* is a gram positive cocci, food used not only for sticking to the dental, but also for synthesis, consequently detect acids. and allows low pH of the bacterium, preventing competitive bacteria from colonizing and eventually causing early decays [6] . Usually, the appearance of *S. mutans* in the tooth cavities is followed by caries after 6-24 months [7] .

In the bacteria, two component regulatory systems (TCRSs) are used as "molecular switches" in the response to ecology changes. regulatory genes are essential for the bacterial adaptation, survival, and the virulence. Basically on the genome sequence, 13 TCRSs genes have been; identified in the *S. mutans* [8]. One of these TCRSs, the *vicR K* signal transduction system, the affects different virulence features of *S. mutans* [9] . This system is consist of a histidine kinase sensor protein (*vicK*) located in the bacterial membrane and a cytoplasmic; response regulator protein (*vicR*). During phosphorylation reactions, extracellular signals is sensed via *vicK*, and then the *vicK* histidine kinase transmits the message to *vicR*, which is modulates gene expression [10] . *VIC* genes regulate expression of several virulence associated genes that is affect produce and adhesion to polysaccharides, including *gtfB*CD, *ftf*, and *gbp*B. Furthermore it, compared with wild-type UA159 strain, strains without *vicK* form thumping biofilms, with a decrease rate of total format ion glucan [11] . In addition, inactivation of *vicK* action a decrease level of lactic acid and best acid tolerance of *S. mutans* [10] ; a *vicK* knockout mutant have been present to be more sensitive to H2O2 than wild-type [12] .

A *vicR* null mutation is present lethal to *S. mutans*. *vicR* acts directly on promoter regions of the *gtf*B, *gtf*C, and *ftf* genes. Over production of *vicR* transcript up regulates these genes [11] . In addition, *vicR* binds specifically to; the comC gene, subsequently negatively affecting the transcription of comC, comDE, comX, and nlmC [13] . The *vicR K* signal transduction system are basic for *S. mutans* by modulating gene expression. sequenced the *vicR* and *vicK* genes of the *S. mutans* strains isolated from; children with a distinct caries status to analyse effects of *vicR* and *vicK* polymorphisms on risk of Early childhood caries (ECC). Mutational analysis present that *vicR* in *S. mutans* plays an basic role in the viability *vicR* , *vicK* of this bacterium [11]

Matrial & Method

In this study, the samples was collected from from oral cavity of human during period from December, 2018 to May, 2019 [AL-Mamoon Specialized Dental Center and Yarmouk Health Center for Family Medicine in Baghdad city]. which sample collection include (7 female and 15 male) both gender 22 from clinical *S. mutans* strains were isolated from caries- children to sequence the *vicR, vicK* and *16S rRNA* genes. Genomic DNA was extracted from *S. mutans* strains and amplified using Gradient PCR. The PCR products were purified and then sequenced. The *vicR* of gene Sequence by specific primer Forward 5'CGGGATCCATGAAGAAAATTC TAATCGTTGACGA- 3', Revers 5'- CCGCTCGAGTT AGTCATATGATTTCATG TAATAAC- 3', 708 base pair [14] The optimum condition of *vicR* gene detection Initial Denaturation 94°C 5 min 1cycle, Denaturation 94°C 45 sec 35 cycle , Annealing 48°C 45 sec 35 cycle, Extension 72°C 7min 1cycle. and specific primer *vicK* of gene Sequence Forward5'- C G G G A T C C A T G A C T A A T G T G T T T G A A TCAAGTC-3', Revers 5 ' - C C G C T C G A G T C A T G A T T C G T C TTCATCTTCTTCC-3', 1353 base pair [14] The optimum condition of *vicK* gene detection Initial Denaturation 94°C 5 min 1cycle , Denaturation 94°C 30 sec 35 cycle, Annealing 58°C 30sec 35cycle, Extension 72°C 6 min 1cycle , the specific primer *16S rRNA* of gene of *S. mutans* Sequence Forward 5'- AGAGTTTGATCCTGGCTCAG- 3' Reverse 5'- GGTTACCTTGTTACGACTT- 3' , 1250 base pair The optimum condition of *vicR* gene detection Initial Denaturation 94°C 3 min 1cycle , Denaturation

94°C 45sec 35 cycle , Annealing 56°C 45 sec 35 cycle, Extension 72°C 7min 1cycle.

Result & Discussion

A total of 22 dental caries in (3-17) age who attending to AL-Mamoon Specialized Dental Center and Yarmouk Health Center for Family Medicine in Baghdad city.

Genomic DNA Extraction The result of this study indicates that extracted DNA from bacterial cultuer sample dental caries was done

Detection of *vicR* gene

vicR gene was ammplifid by Gradient PCR and then gel electrophoresis was verified by 1.5 % (w/v) agarose using a ladder (1500) as a molecular weight marker.

Figure 1: Analysis of *vicR* gene product was electrophoresis on agarose gel (1.5 %) at 70 volt. 1x TBE buffer for 1 hours. *vicR* product band size (708 bp). Lane(N): DNA Marker sizer (1500) and lane 1-22 for sample denal caries.

Sequencing of 708 bp amplicons of *vicR* gene

Eight samples was sent to Korea sequencing which had shown exactly 708 bp The sequencing reactions indicated the exact positions after performing NCBI for these PCR amplicons. Concerning the supposed 708 bp PCR amplicons of *vicR* gene, NCBI engine has shown extremely high sequences similarities between the sequenced samples and this target. NCBI (http:// www.ncbi.nlm.nih.gov) engine has indicated the presence of 100% of homology with the expected target that completely covered

appear analysis sequencing of *Streptococcus mutans vicR* gene no mutation because highly conserved among the clinical isolates

Detection of *vicK* gene

vick gene was ammplifid by Gradient PCR and then gel electrophoresis was verified by 1.5 % (w/v)

agarose using a ladder (1500) as a molecular weight marker.

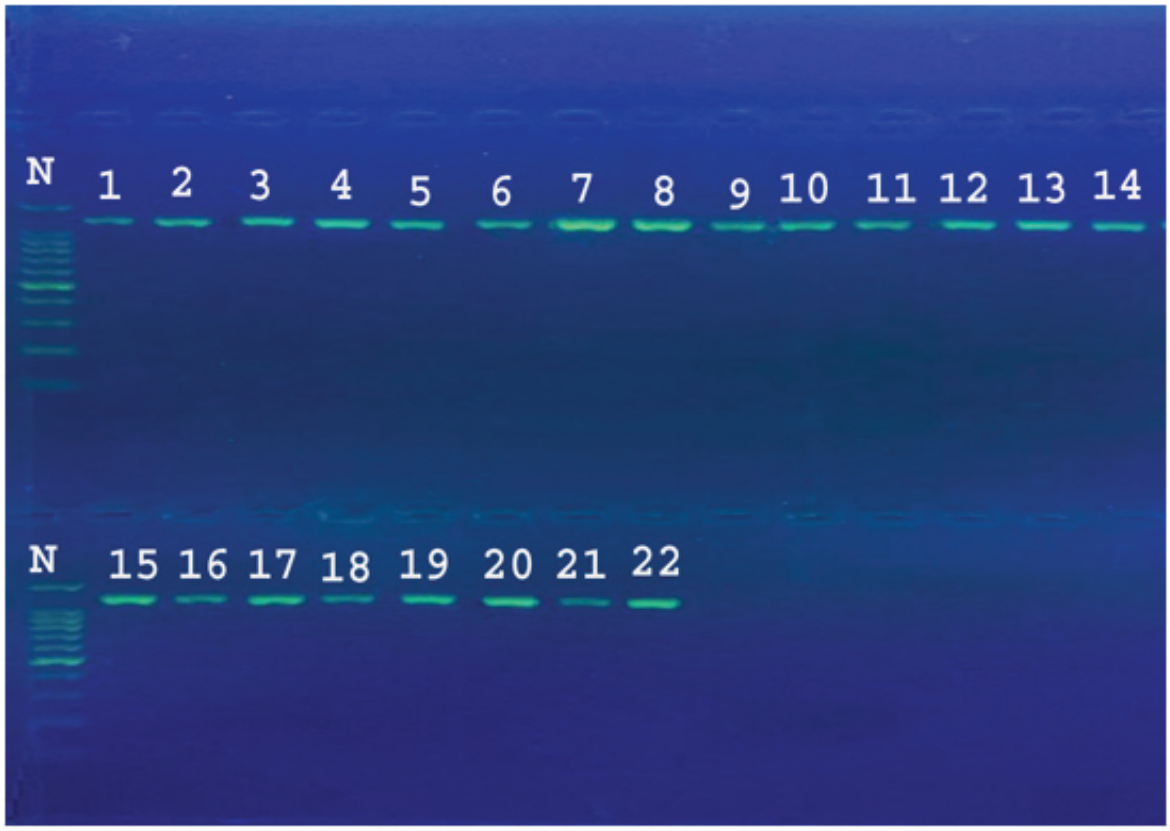

Figure 2 : Analysis of *vicK* gene product was electrophoresis on agarose gel (1.5 %) at 70 volt. 1x TBE buffer for 1 hours. *vicK* product band size (1353 bp). Lane(N): DNA Marker sizer (1500) and lane 1-22 for sample denal caries.

Sequencing of 1353 bp amplicons of *vicK* gene

Eight samples was sent to Korea sequencing which had shown exactly 1353 bp The sequencing reactions indicated the exact positions after performing NCBI for these PCR amplicons. Concerning the supposed 1353 bp PCR amplicons of *vicK* gene, NCBI engine has shown extremely high sequences similarities between the sequenced samples and this target. NCBI (http:// www.ncbi.nlm.nih.gov) engine has indicated the presence of 99% of homology with the expected target that completely covered

Table (1) analysis sequencing of *Streptococcus mutans vicK* gene

Type mutation	Nucleotide change	Amino acid change	Isolation
silent mutation	AAA>AAG	Lysine> Lysine	**All isolation**
	GAA>GAG,	Glutamic acid> Glutamic acid	
	AAT>AAC	Asparagine> Asparagine	
	ACC>ACT	Threonine> Threonine	
	GTA>GTT	Arginine> Arginine	

Cont... Table (1) analysis sequencing of *Streptococcus mutans vicK* gene

missense mutation	GGT>CGT, AAT>AAA, CAG>CAC CAG>CGG	Glycine > Arginine Asparagine> Lysine Glutamine> Histidine Glutamine> Arginine	**2,3,7**
	TTT>TGT CAA>CAT AAA>ACA ATG>ATA	Phenylalanine > Cysteine Glutamine> Histidine Lysine> Threonine Methionine> Isoleucine	**4**

This table 1 appear that most of mutation was silent mutation in all bacterial isolates that mean the mutation change to the same amino acid change may well have little effect on the protein sice the substituted amino acdis are similar to the original (they are all hyphobioc amino acid) such as Lysine> Lysine AAA>AAG, Glutamic acid> Glutamic acid GAA>GAG, Asparagine> Asparagine AAT>AAC, Threonine> Threonine ACC>ACT, Arginine> Arginine GTA>GTT But four isolates (2,3 and 7) was give a missense mutation, Glycine > Arginine GGT>CGT, Asparagine> Lysine AAT>AAA, Glutamine> Histidine CAG>CAC, Glutamine> Arginine CAG>CGG, this mutation may be cause high biofilm product, (benefint mutation or positive mutation) because can be increase the biofilm product adhernace. And one isolates No. (4) was give missense mutation Phenylalanine > Cysteine TTT>TGT, Glutamine> Histidine CAA>CAT, Lysine> Threonine AAA>ACA, Methionine> Isoleucine ATG>ATA, this mutation cause decrease biofilm produce, (harmful mutation or negative mutation**),** mutation lead to decrease production biofilm

Detection of *16S rRNA* gene

16SrRNA gene was used to increase the confirmation of bacteria *S.mutans* deposite begin diagnosis by GP 24 kit, *vicK* gene detected by electrophoresis Amplification was verified by electrophoresis 1.5 % (w/v) agarose using a ladder(1500) as a molecular weight marker. An amplification of *16S rRNA* from 22 isolates was performed to confirm bacterial identification, Identification of S. mutans isolates by using 16S rRNA is more accurate than bacteriological and biochemical assays. [15] demonstrate that 16S rRNA gene PCR was sensitivit, specific,and used for diagnosis of culture-negative bacterial Infections also useful for identification of bacterial pathogens in patients pretreated with antibiotics.

Figure 3 : Analysis of *16S rRNA* gene product was electrophoresis on agarose gel (1.5 %) at 70 volt. 1x TBE buffer for 1 hours. *16S rRNA* product band size (1250 bp). Lane(N): DNA Marker sizer (1500) and lane 1-22 for sample denal caries.

Sequencing of 1250 bp amplicons of *16S rRNA* gene

Ten samples was sent to Korea sequencing which had shown exactly 1250 bp The sequencing reactions indicated the exact positions after performing NCBI for these PCR amplicons. Concerning the supposed 1250 bp PCR amplicons of *16S rRNA* gene, NCBI engine has shown extremely high sequences similarities between the sequenced samples and this target. NCBI(http:// www. ncbi.nlm.nih.gov) engine has indicated the presence of 98- 99% of homology with the expected target that completely covered.

In this appear *16S rRNA* gene despite the emergence of mutations in all isolates they gave a ratio 98-99% *S.mutans* The sequence was blasted in NCBI against standard strain of S. mutans complete genome. The identifying result showed 98-99%.

Conclusion

The result of molecular study of *vicR* gene were appear no mutation in all isolates while *16S rRNA* although have transition and transvertion mutation but the percentage of similarty with NCBI (http:// www. ncbi.nlm.nih.gov) appear 98-99% that Streptococcus mutance partial *16S rRNA* gene. While the Sequence of *vicK* gene that note more of mutation was silent mutation but missense mutation present in isolated that cause change in biofilm production.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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