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The Role Of Micorna 146-A (Rs2910164) Among Otitis Media Patients

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Abstract: The current study aims to show the relationship between gene polymorphisms (SNP) of miRNA 146-a of patients infected with otitis media. This finding included 50 samples collected from healthy subjectss and 100 samples from patienst suffering from otitis media who attended Al-Sadr Medical City (ENT Department) in Al-Najaf Governorate from February 2022 to June 2022. The samples had an average age ranging from 5 to 70 years. Genotyping of miRNA 146-a revealed the presence of three genotypes: GG, CG, and CC, representing (16%, 47%, and 37%) respectively in patients with otitis media, while it was (27%, 18%, and 55%) respectively in healthy subjects. CG was common in patients while GG was more common in healthy subjects—miRNA as a potential biomarker of patients infected with otitis media.

Keywords: Genotyping ,miRNA146-a ,Otitis Media , ENT, GG, CG

1.Introduction

Otitis media is an inflammation of the middle ear that is recognized by tympanic membrane and middle ear inflammation resulting from the infection of upper respiratory tract (URT) [1]. It is known as one of the most common infections of childhood because of the anatomically horizontal and shorter Eustachian tube so the pathogens can enter more easily from the nasopharynx and then cause the infection [2]. Otitis media often is the leading cause for antibiotic resistance in the development world. The diagnosis of otitis media should be based on symptoms presence of middle ear effusion, and signs of ear inflammation with a bulging of the tympanic membrane [3].

There are three types of otitis media: (AOM) Acute otitis media is defined as the presence of effusion in the middle ear with one or more symptoms or signs of inflammation of the middle ear[4]. And is the most popular bacterial infection and cause of antibiotic use in early childhood[5]. Acute otitis media may lead to cumulation of chronic fluid in the middle ear so the tympanostomy tube insertion (TTI) is the recommended treatment [6] ,and is the most popular surgical procedure implemented on children[7].Children are prone to developing acute otitis media because of immunological and anatomical immaturity, whereas adult ear infections are typically chronic [8].

The recurrences of (AOM) may lead to Otitis media with Effusion (OME), which is a chronic form of Otitis media without symptoms or signs of Acute otitis media, and the

tympanic membrane is not perforated ,the local inflammation leads to a collection of liquid in the middle ear cavities and epithelial changes (metaplasia),the effusion nature is mucous or seron-mucous but not purulent , lasts for at least three months,this set is apart from the persistent effusion after(AOM), which disappear after two months in 90% of the cases[9].

The third type is (CSOM) Chronic suppurative otitis media, which is persistent perforation in the tympanic membrane with recurrent Otitis media and is a chronic inflammation of the mastoid cavity and middle ear recognized by drainage of liquid from the ear (otorrhea) persisting for at least (2-6 weeks)through a perforate in the tympanic membrane [10].

The new frontier in the regulation of gene expression is represented by microRNAs which are small molecular RNA of about 22 nucleotides,that could regulate gene expression by binding with 3'-(UTR)untranslated region of the target gene, .which play a vital role in inflammation,immune response and metabolic disorder[11]. Changed miRNA expression cause alteration in the expression of some important genes . Polymorphism in the miRNA may lead to individuals genetic susceptibility to certain inflammatory diseases[12]..Polymorphisms of genes could indicate the disease's development. Polymorphisms identification may help clinicians to indicate the disease's development and help to conclude the genetic sensitivity of the population. Polymorphisms identification may help clinicians choose the right treatment for the patients [13,14,15]. .MiRNA may regulate about 30% of human genes and expression patterns are greatly comparable between healthy and patient persons [16].

2.Methodology

Subjects: This case-control study used 150 clinical samples, 90 males and 60 females, with ages ranging from (5 to 70). It was

conducted between February 2022 and June 2022. The first group was patients with discharge Otitis media (100), males (68) and females (32), First, patients were personally questioned by a researcher using an anonymous questionnaire form that included (age and gender).The second control group, 50 randomly selected healthy people (5–70)years old, (30 males, and 20 females) . This study agrees with the same ethics as patients admitted to the Al-Sader Teaching Hospital in Iraq's Al-Najaf Governorate.

Each subject's venous blood was collected for five millilitres. Before the blood was taken, a tourniquet was placed immediately on the skin around the arm, and the skin around the vein was sterilized with 70% ethyl alcohol from the patients and control group. The blood are taken in sterile tubes containing EDTA for DNA and RNA extraction, followed by the use of the (Real-time PCR) technology. These samples should be immediately frozen at -20C .

Table (1) show the primer miRNA 146a (rs2910164) of patients with Otitis media

Genes	Primer sequence(5'-3')	(pb)	R e f
miRNA 146a (rs2910164)	Primer-F (Allele-C) 5'- TCCATGGGTGTGTC AGTGTCAGAGCTC-3' Common Primer-R 5'- GAGTAGCAGCAGCA GCAAGAGAGACTT3' Common Primer-F 5'- TAGACCTGGTACTA GGAAGCAGCTGCAT- 3' Primer-R (Allele-G) 5'- ATATCCCAGCTGAA TTACAC-3'	290bp	[1 7] .
		203bp	

3.Results and Discussion

Genotyping of miRNA 146-a

This case-control research, and genotyping of the miRNA146-a (rs2910164) polymorphism among those suffering from Otitis media and healthy individuals. Using a specific primer (miRNA146-a), tetra-primer amplification refractory mutation system-

polymerase chain reaction (T-ARMS-PCR), SNP of the miR-146a gene (rs2910164 C/G) were evaluated. Polymerase chain reactions were carried out to amplify the isolated DNA from each sample for the patient and control groups. The results of the miRNA146-a polymorphism detections as shown in Figure(1) displayed the results of amplified PCR product gel electrophoresis; different band sizes compared to DNA ladder bands were obtained, a locus reveals three genotypes: CG, GG, and CC with bands size (445,290 and 203) for CG ,(445 and 203) for GG and (445 and 290) for CC .

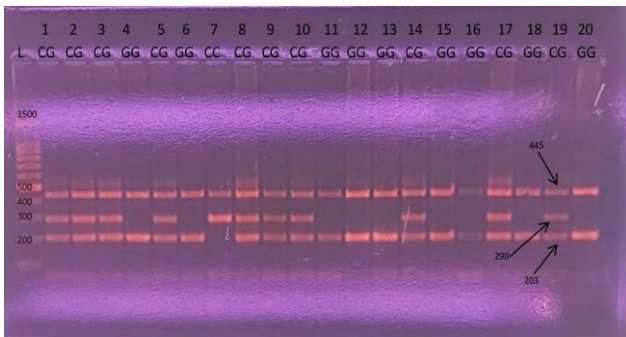


Figure (1) -Agarose gel stained with ethidium bromide displaying the TARMS-PCR results for the miRNA 146-a (rs2910164) polymorphisms, 1 (CC genotype), 10 (CG genotype), and 8 (GG genotype).

The following genotypes were shown to be more common in OM patients: CC (16%) ,CG (47%) and GG (37%) ;while in the control ;CC (27%) ,CG (18%) and GG (55%) , Table (2) .The same tTable explainedThe same Table explains that allele G was high frequency and showed significant differences between CG genotypes at P-value ≤ 0.05 .

Table (2) miRNA146-a rs(2910164) genotype and allele frequency in OM patients

Mi RNA 146-a	Patient n=38	Control n=22	OR(95 % CI)	P-value
C/C	(6) 16%	(6) 27%	0.500 (0.138 to 1.799)	0.288 NS
C/G	(18) 47%	(4) 18%	4.050 (1.152 to 14.231)	0.029 *
G/G	(14) 37%	(12) 55%	0.486 (0.167 to 1.413)	0.185 NS
Allele frequency				

C allele	30	16	1.141 (0.529 to 2.458)	0.735 NS
G allele	46	28	0.876 (0.406 to 1.887)	

OR : Odds Ratio , CI : Confidence interval , *(P<0.05): Significant

Our study analyses the association of miRNA146-a gene polymorphism with OM. There is currently no evidence linking the polymorphism of miR-146a to otitis media illness. As a result, we conducted an evaluation study to determine the association between OM and polymorphisms of miRNA-146a (rs2910164).

MicroRNAs (miRNAs) are a group of single-stranded noncoding RNA molecules present in plants, animals, some viruses, and other life forms, including about 22 nucleotides . .Small-interfering RNAs and miRNAs are related in many ways. Including (RNA silencing and translation suppression) which are two of miRNAs' functions [18]. .Previous research reported that miRNAs had a role in various intricate biological processes, including cell differentiation, apoptosis, development, proliferation, and others [19]. .

Increasing evidence suggests miRNAs may control the expression of many important genes. .According to a recent study by Shin and Chu, miRNAs may serve as significant biomarkers and potential targets of therapy for CG[21]. One important regulatory component for the immune system has been identified as control of miRNAs[22]. Additionally, it has been shown that microRNAs play a significant role when various pathogens, such as viruses, parasites, and bacteria, invade a host It has been suggested that miRNAs may play an important role in the immune response to intracellular and extracellular infections such as Staphylococcus aureus, Pseudomonas aeruginosa, and Burkholderia spp [23]..

Single nucleotide polymorphisms (SNPs) ,are mutations in the miRNA sequence that may impact miRNA expression or maturation [24]. The rs2910164 polymorphism, which results from a nucleotide change from guanine (G) to cytosine (C), is a function variant of miRNA146a. Studies revealed that

the rs2910164 polymorphism's C allele significantly raised the levels of the miRNA-146a protein [25].

Additionally, a number of case-control studies and functional analyses revealed that miRNA SNPs might alter CG susceptibility and that this impact was directly tied to their function in miRNA expression[26]. . Studies have demonstrated that genetic changes (SNPs) in miRNA sequences can affect how primary miRNAs (pri-miRNAs) and precursor miRNAs (pre-miRNAs) are processed, changing how miRNAs express themselves[27]. According to earlier studies, the SNP rs2910164 at the pre miR-146a (C/G) exhibited a substantial association with several diseases [28].

According to Jazdzewski *et al.*, functional single nucleotide polymorphism (SNP) variants inside specific miRNAs may impact how the pre-miRNA is processed to become the mature form of the miRNA[29]. For example, C could convert into G and affect how well miRNAs regulate mRNAs [29]. Several studies conducted that miRNA genetic mutations significantly impact how the immune system develops and responds, and they may lead to autoimmune, inflammatory, and cancer diseases [30].

Our findings showed that at position rs2910164, CG genotype differed significantly between the case and control groups. Still, there was no significant difference in the allele frequencies of rs2910164 between the case and control groups. Numerous investigations have shown that there was no appreciable difference in the distribution of the miR-146a (rs2910164 CG) genotypes between the patient and control groups in several diseases, including TB, gastric cancer, coronary artery, and colon cancer [31].

Researchers in China looked at how miRNA polymorphisms changed in response to the hepatitis B vaccine antibodies and discovered a significant difference in the frequency of the location of rs2910164 between the responders and non-responders. Additionally, in comparison to the other

genotypes, the CC genotype produced a 1.74-fold rise[32]. Shao *et al.* demonstrated that the C allele and the CC genotype were more common at position rs2910164 in sepsis [33]. In a prior investigation of Zhang *et al.* substantial differences in the allele G and CC genotype of the miR-146 C>G SNP were discovered between patients with pulmonary tuberculosis and healthy controls in the Kazak community[34]. Al-Omari, and Al-Ammar, show the Relationship of IL-6 gene polymorphisms and IL-6 expression level with the burn-induced sepsis susceptibility in Al Diwanayah [35].The research of Al-Hammami & AL-Ammar demonstrated that the frequency of the miR-146a alleles and genotypes did not differ significantly in the Uyghur populations [35].

4.Conclusion

The study that investigated the genetic polymorphism (SNP) miRNA 146-a (rs2910164) that related with Otitis Media. The presence of significant differences in CG genotype and G allele from miRNA146 -a (rs2910164) in OM patients may be used to predict this disease.

Ethics:

This study was conducted under approval by the medical ethics committee at the University of Kufa (2017). Parents and agreement provided verbal and written consent for publication was obtained from both participants and researchers.

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