

<https://doi.org/10.48047/AFJBS.5.4.2023.75-85>



African Journal of Biological Sciences



Open Access

Research Paper

Estimate levels of beta defensin1 and matrix metalloproteinase in chronic otitis media infection

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Article Info

Volume 5, Issue 4, October 2023

Received:14 Aug 2023

Accepted :19 Sept 2023

Published: 12 Oct 2023

doi:10.48047/AFJBS.5.4.2023.75-85

Abstract

Background: chronic suppurative otitis media is a long-standing and persisting draining perforation usually caused by acute otitis media, blockage of the Eustachian tube. It may flare up following nose or throat infection.

Objectives: The study aimed to explore the ratio of matrix metalloproteinase, and Beta defensin1 in patients with otitis media infection and compare them with healthy individuals. **Materials and methods:** Initially, sixty peripheral blood samples were taken from patients of both gender with different age groups attending ENT clinic and Kirkuk General hospital at Kirkuk city besides, thirty samples were obtained from healthy individuals as a control group. The estimation were conducted using Elisa technique and the data was statistically analyzed according ANOVA standards of Variance procedure depending on duncan's multiple range coeفتهint .

Results: the current work revealed the highest percentage of infections according to the age of 21 -40 years recording 35%. Male patients had a privilege with infection 58% more than female. The results showed an increase in matrix metalloproteinase MMP13 with a mean recording 457.0 ± 73.00 compared to health people which was 333.5 ± 56.62 The result gave an increase in beta defensin1 recording 42.52 ± 6.59 compared to healthy individual which was 30.3 ± 5.82 with a significant differences for both. The study also reveal positive relation among those two variables with significant correlation at the 0.01 level **Conclusion:**. The photolytic metalloproteinase enzyme as well as β defensin increased highly among chronic patients and had positive association so they could be good indicators for the follow up and recovery.

Keywords: matrix metalloproteinase, β defensin1, Chronic suppurative otitis media.

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Introduction

Chronic suppurative otitis media (CSOM) is a prevalent ear infection in humans, affecting approximately 65-330 million individuals annually. Children, particularly school children, are more vulnerable to this infection compared to adults. The condition involves persistent inflammation in the middle ear and mastoid cavity, as well as a tympanic membrane perforation and the presence of purulent discharge^(1,2). Typically, the inflammation arises following an Acute otitis media or upper respiratory infections. Various microorganisms demonstrate an increased incidence of the disease, including bacterial strains like *E. coli* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Proteus* spp. which are responsible for causing otitis media. External factors contribute to the spread of the infection in the populace, such as personal hygiene, congestion, hunger, and passive smoking exposure^(3,4). MMP-13 has been found in both normal epithelial and neural cells.⁽⁵⁾ Nevertheless, MMP-13 expression and secretion in normal human tissues remain low and strictly regulated by several variables. MMP13 is a member of a broad family of zinc-dependent neutral endopeptidases that can degrade the extracellular matrix. MMP13, known as collagenase, plays a critical role in breaking down in vivo natural collagen fibrils, and it is believed to have a rate-limiting function in extracellular matrix remodelling that is required for morphogenesis, tissue remodeling, and corneal wound healing.⁽⁶⁾ MMP13 (collagenase-3) is the only collagenase that can cleave type I, II, and III collagens. can also cleave Types IV, X, and XIV collagen⁽⁷⁾. Due to its broad MMP13 expression is dependent on substrate specificity. restricted to physiological circumstances where Rapid and efficient remodeling of the collagenous extracellular matrix is required.⁽⁸⁾ MMP13 is found in the cornea. expression is Only the basal layer of repairing corneal epithelium was observed.⁽⁹⁾ Its involvement in altering the underlying basement membrane is suggested. Furthermore, excessive expression and/or MMP13 activation have been associated with the breakdown of extracellular matrix in conditions like Chronic cutaneous ulcers, intestinal ulcerations, and chronic periodontitis are all symptoms of osteoarthritic cartilage⁽¹⁰⁾.

Numerous MMP13-specific inhibitors have been developed to address without the osteoarthritis and rheumatoid arthritis typical adverse effects of nonselective MMP inhibitors⁽¹¹⁾. Additionally, these inhibitors hold possibility of mitigating tissue deterioration and ulceration induced by infections. hBD1 (human-defensin-1) exhibits antiviral efficacy against enveloped as well as non-enveloped viruses⁽¹²⁾. as encoded by DEFB1 gene, hBD1 is created by various cells produced from epithelia and bone marrow and possesses antibacterial activity in the presence of diverse Bacteria, viruses, fungus, and protozoa are examples of pathogens.⁽¹³⁾, as well as several pathogenic bacteria. Different AMPs (Antimicrobial Peptides) target distinct bacterial sites or employ various mechanisms of activity, as recently reviewed by others⁽¹⁴⁾. Human genome studies have identified over 28 β -defensin genes⁽¹⁵⁾. Epithelial cells on mucosal

surfaces of the stomach, skin, airway, mouth, kidney, nose, eyes, mammary glands, and female and male genital tracts are the primary sources of HBD1 expression. ⁽¹³⁾. Epithelial cells manufacture it on their own., HBD1 is considered a possible mediator of lower respiratory tract mucosal immunity ⁽¹⁶⁾. It is also found in the lungs' respiratory epithelium., providing protection against respiratory pathogens⁽¹⁷⁾. Given that viral infections of the respiratory tract contribute significantly to human morbidity and mortality, hBD1 has demonstrated antiviral action against influenza A virus (IAV), respiratory syncytial virus (RSV), and rhinovirus (RV)⁽¹⁸⁾. These peptides' antiviral activity is mediated by different methods. including direct binding of the virus to the peptide, indirect inactivation through modulation of viral replication or signaling cascades, as well as immune cell recruitment ⁽¹⁸⁾.

Materials and Methods:

Blood samples from individuals of various ages were gathered, totaling sixty samples, at Kirkuk General Hospital from the Ear, Nose, and Throat Unit. Additionally, thirty healthy samples were also obtained. Following the collection of whole blood, the samples were left undisturbed at room temperature to allow clotting, which typically It takes between 10 and 20 minutes. The clot was then removed by centrifuging it for 20 minutes at 2,000-3,000 rpm. If any precipitates were observed during this process, the sample underwent centrifugation again to obtain serum.

Ratio estimate of matrix metalloproteinases13 and beta defensin1

This ELISA kit employs the Sandwich-ELISA method. The stripplate supplied by Microelisa with the kit comes pre-coated with an MMP-13-specific antibody. To perform the test, Standards or samples are placed in the appropriate wells. of the Stripplate for microelisa, where they bind to the particular antibody. Next, Each well is treated with a Horseradish Peroxidase (HRP)-conjugated antibody specific for MMP-13. After incubation, The unneeded components are rinsed away.. Subsequently, Each well receives the TMB substrate solution. Only those boreholes containing both MMP-13 and HRP-conjugated MMP-3 antibodies will be used exhibit a the color blue, which turns yellow upon addition of the stop solution. The optical density (OD) is defined as then At 450 nm, it was detected spectrophotometrically. The OD measurement is directly proportional to MMP-13 concentration. By comparing the OD relating to the samples standard curve, you can calculate the concentration of MMP-3 in the samples. The same method was used to estimate beta defensin1.

Statistically analyzed

The anova procedure is used, along with Duncan's multiple range test for Y1 using the multiple range function. It's essential to note that this test

specifically controls the Type I comparisonwise error rate, rather than the experimentwise error rate.

Results

Out of the 60 isolated samples from patients with chronic otitis media, 5 different strains of bacteria were identified. These strains include 3 types of gram-negative bacteria: *Pseudomonas aeruginosa*, *Escherichia coli* (*E. coli*), *Proteus vulgaris*, Additionally, two species of gram-positive bacteria were found: *Staphylococcus aureus* and *Staphylococcus epidermidis*. The highest percentage of infections, at 35%, was observed in the age group of above 21 -40 years. The incidence of men was higher than that of women. Rate of man 58%.rate women 42%

Evaluation of Matrix metalloproteinase -13 (Mmp-13)

The present study revealed a higher concentration of MMP-13 in individuals with otitis media (457.0 ± 73.00) compared to healthy individuals (333.5 ± 56.62). Our findings demonstrate that serum MMP-13 levels are elevated in both otitis media patients and healthy individuals.

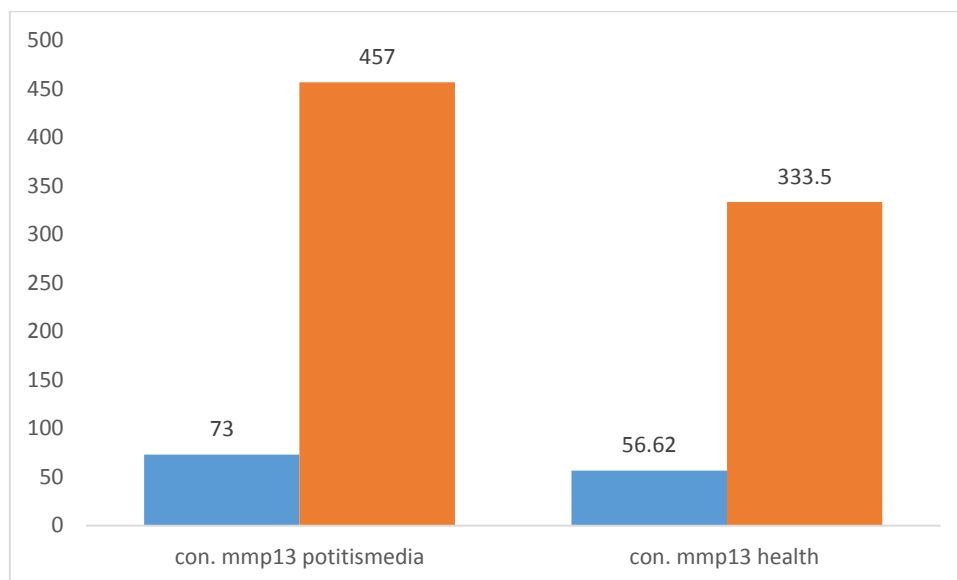


Fig : The percentage of healthy and injured

The findings are consistent with previous studies ^(19,20), which reported a significant increase in serum MMP-13 levels among patients with otitis media compared to healthy individuals. MMP-13, also known as collagenase 3, is an interstitial collagenase that is prominently expressed in cancers and cancer stromal cells ⁽²¹⁾. Its collagenolytic activity extends not just to collagens I, II, and III, but also to collagen IV encompasses a diverse range of extracellular matrix (ECM) elements. The regulation of MMP-13 expression and secretion occurs at both the transcriptional and cellular levels⁽²²⁾. Substantial evidence supports the

notion that degradation mediated by MMP-13 and remodeling of the ECM play a crucial etiology and metastasis of cancer .⁽²³⁾

Evaluation of β defensin1

In the present study, the concentration of beta-defensin in otitis media was measured at 42.52 ± 6.59 , which was higher compared to healthy individuals, where it measured 30.3 ± 5.82 . Our findings indicate an increased serum concentration of beta-defensin in both otitis media patients and healthy individuals.

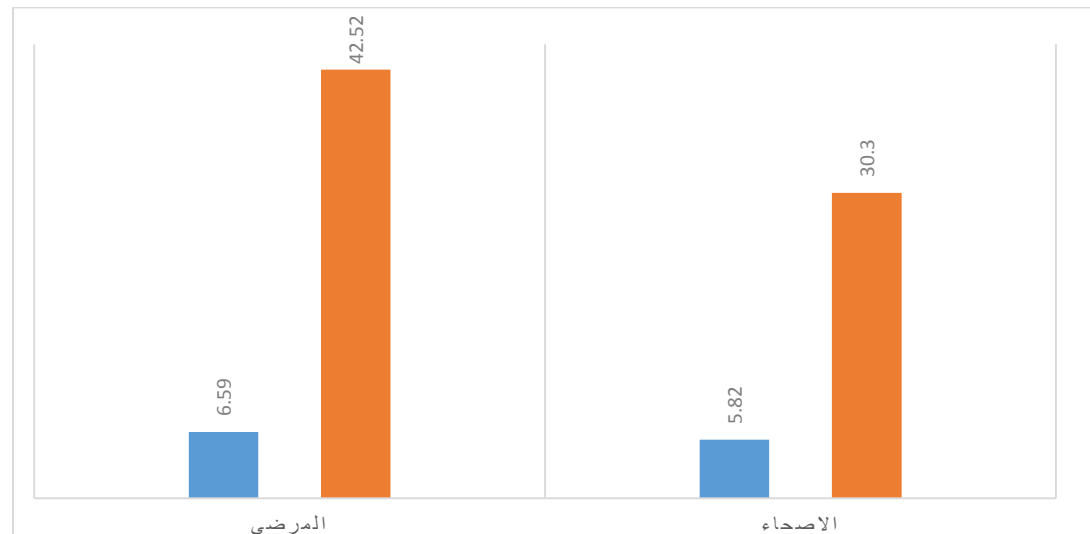


Fig : The percentage of healthy and injured

The results are in agreement with the findings from reference ⁽²⁴⁾. Our previous study demonstrated that hBD1 is capable of display Under reducing circumstances, hBD1 had wide antibacterial action, whereas only E. coli was impacted by the oxidized form of hBD1 (hBD1ox)⁽²⁵⁾. The persistence antimicrobial peptides produced by endogenous host defense and their lack of evoking resistance, particularly in situations of constant use, has been a topic of discussion since their discovery. For quite some time following the identification of hBD1, researchers pondered over why would an antibacterial peptide be useful exhibited such limited Antimicrobial activity. HBD1 is most likely one of the most abundant antimicrobial peptides known, being expressed not just by all epithelial surfaces but also by circulatory and reproductive cells.. Interestingly, hBD1 polymorphisms are linked to an inability to effectively clear potentially harmful MRSA and other microorganisms .⁽²⁶⁾

Correlation between hBD1 and mmp13

The association between hBD 1 and MMP13 was analyzed for highly significant results as shown in the table 4.6

| | B-def1 | MMP13 |
|--|--------|-------|
| B def1. pearson correlation sig.(2-tailed) | 1 | .343 |
| N | 60 | 60 |
| MMP13 pearson correlation sig.(2-tailed) | .343 | 1 |
| N | 60 | 60 |

The correlation is statistically significant at the 0.01 level (two-tailed).

The study showed that an increase in human beta defensin1 peptide increased in the case of bacterial infection in the middle ear along with increases in matrix metalloproteinase . This result agreement TLR2 detects lipoproteins and LTA from Staphylococcus aureus and promotes the release of AMP b-defensin 3, IL-1 family cytokines, chemokines (C-X-C motif) ligand (CXCL) 1 and CXCL2. Mice lacking TLR2 are more susceptible to S. aureus infection. S. aureus phenol-soluble modulin (PSM) peptides mobilize lipoproteins for recognition by TLR2.⁽²⁷⁾ TLR2 also recognizes Propionibacterium acnes in human keratinocytes by heterodimerizing TLR2/1 and TLR2/6, activating NF-kB and activator protein 1 ⁽²⁸⁾ TLR5 triggered by bacterial flagellin increases the expression of AMP S100A8/S100A9, S100A7, S100A15, and human b-defensin (HBD) 2 in keratinocytes.TLR5 detects flagellins from Treponema pallidum and activates matrix metalloproteinase (MMP) 9 and MMP13 via the MAPK/NFkB signaling pathway. TLRs help the skin defend itself against fungi and viruses in addition to bacteria. Keratinocytes interacted with Malassezia spp. most likely through TLRs. ⁽²⁹⁾ so far only a few studies applied Correlation between hbd1 and mmp13

Discussion

1. The results of injury in men are higher than that of women and are confined between the ages of 21 to 40, as a result of exposure to explosions, smoking or swimming
2. There was an increase in the enzyme matrix metalloproteinases as a result of infection with bacteria, compared to infected and healthy subjects.
3. There was an increase in beta defensin enzyme as a result of infection with bacteria compared to infected and healthy subjects
4. There was a positive relationship between matrix metalloproteinases and beta defensin

Ethical considerations

This work research was carried out in accordance with the ethical guidelines. The objective of this study was verbally conveyed to the participants. Prior to collecting any samples, ethical approval was obtained and patients were selected for inclusion in the study. The goal and process of the survey were effectively communicated by the researcher to the patients, accompanied with standardized instructions and guidance for the completion of the questionnaire. The study design, patient information, and permission form underwent a thorough evaluation and received approval from the local Ethics Committee

No.12960 on 9th October 2022

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Cite this article as: Saif Ali Hussen (2023).

Estimate levels of beta defensin1 and matrix metalloproteinase in chronic otitis media infection. African Journal of Biological Sciences. 5(4), 75-85. doi: 10.48047/AFJBS.5.4.2023.75-85