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Original Research Article

Biochemical and Molecular Study of Meteorin-Like Protein in Atherosclerosis Patients

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Name of Author:
Aser Ihsan Abdullah[1*] and Saba Zeki Al-Abachi[2]

Affiliation: *[1]University of Mosul, College of Environmental Science, Department of Environmental Science, Mosul, Iraq*
[2]University of Mosul, College of Science, Department of Chemistry, Mosul, Iraq

Corresponding Author:
Aser Ihsan Abdullah

Abstract: ***Background***: Meteorin-like protein (Metrnl) is a newly discovered cytokine secreted by skeletal muscle and adipose tissue, recently studies have described its potential role regulating inflammation and metabolism, gave us an impression of his contribution in vascular pathology and cardiovascular disease particularly atherosclerosis. ***Objectives***: This study was designed to assess its involvement of Meteorin-Like Protein in atherosclerosis and to evaluate the molecular characteristic of Metrnl gene in atherosclerosis patients in Mosul city by assessing the level of gene expression and DNA sequence results matching. ***Materials and Methods***: 180 blood sample was collected from individuals aged 35–75 years, including patients diagnosed with atherosclerosis and healthy controls for biochemical study and 60 blood sample were collected for molecular study Between January and December 2025. Sandwich kit from BT LAB Chines Company was used to detect Human Meteorin-Like Protein in serum of blood sample; DNA and RNA were extracted from each sample. The purity of Metrnl was evaluated using a Nanodrop spectrophotometer, while gene expression levels were quantified through quantitative PCR, then DNA sequencing was done to determine if there is any variation within the gene sequence, then our finding was submitted in NCBI GenBank data base ***Results***: level of Metrnl is Serum of atherosclerosis patients is significantly decreased (1.322ng/ml) as compared to the healthy control group (3.3132ng/ml) and the ROC curve analysis establish very good diagnostic accuracy, which reveal area under the curve (AUC) of 0.871. The best cut-off value of serum its level that predicted atherosclerosis was 2.2585 ng/ml. The qPCR amplification curves demonstrated that the expression level of Metrnl in the patient group (0.45) was markedly lower than that of the control group (1.0), DNA sequence analysis and matching with the nucleotide sequence of the main gene at (NCBI) show that the Metrnl DNA sequence is exactly identical to Metrnl sequence in the database. The gene was successfully deposited in the GenBank (Accession number: LC874112 (***Conclusion***: Mertrnl rule as atherosclerosis disease progression suppressor, indicated by its significant downregulation in atherosclerosis patients. This notable reduction in expression could influence the severity or progression of the disease. These findings suggest that Metrnl holds potential as a biomarker for diagnosing of atherosclerosis and its complications and may serve as a treatment target.

Keywords: Atherosclerosis; epigenetic; gene expression; Metrnl; Meteorin like Protein, Q-PCR.

INTRODUCTION

Atherosclerosis is a chronic, multifocal inflammatory disease of the arterial vessels and represents the principal underlying cause of heart disease and stroke. It is estimated to account for approximately 50% of all deaths in Western societies [1]. The historical burden of this condition has been highlighted through paleopathological studies, where investigations of mummies dated between 3000 B.C. and 400 A.D.

provided evidence of vascular atherosclerosis in ancient populations [2].

The pathological process of atherosclerosis often begins during adolescence and progresses throughout the lifespan. It is characterized by cholesterol deposition, macrophage infiltration, vascular smooth muscle cell proliferation, and accumulation of extracellular connective tissue within the intimal layer of the arterial wall [3]. The term "atherosclerosis" originates from the Greek words athero (porridge or paste) and sclerosis (hardening), with the suffix -osis denoting a diseased condition [2].

Atherosclerotic cardiovascular disease (ASCVD) develops through a complex interaction between genetic susceptibility and multiple modifiable and non-modifiable risk factors. Assessing the relative contributions of these risks to the overall disease burden is critical for devising effective prevention strategies [4]. In 2021, the global age-standardized rates (ASRs) per 100,000 population for ischemic heart disease (IHD) were reported as follows: 1856.5 disability-adjusted life years (DALYs), reflecting the overall disease burden; 365.7 for incidence; 3042.3 for prevalence; and 110.9 for mortality. Across all metrics, the burden of IHD was consistently higher among males [5].

Regionally, cardiovascular disease remains the leading cause of death in the United Arab Emirates (UAE), responsible for nearly 40% of all registered deaths [6]. Recent findings demonstrate that the UAE has the second-highest prevalence of established ASCVD (eASCVD) in the Middle East, with an overall rate of 29.7% comprising 35.6% among males and 20.5% among females. This is notably higher compared with the regional average of 20.9% (26.6% in males and 16.0% in females), encompassing countries such as Bahrain, Egypt, Jordan, Kuwait, Qatar, and South Africa [7].

Atherogenesis is a multifactorial process that involves several interconnected biological pathways, among which epigenetic modifications have recently gained considerable attention [8]

Emerging evidence suggests that pro-atherogenic factors, such as elevated levels of low-density lipoprotein (LDL) cholesterol and its oxidized derivatives, can induce persistent epigenomic reprogramming within innate immune cells. Such epigenetic alterations, although mitotically heritable, remain pharmacologically reversible, thereby highlighting their therapeutic potential.

In this context, a growing number of researches have been developed in recent years to selectively understand the role of Meteorin-like protein in the pathogenesis and progression of atherosclerosis. Emerging evidence suggests that Metrnl may serve not only as a promising biomarker for monitoring the onset and advancement of atherosclerotic disease but also as a potential therapeutic target. Such findings underscore its dual significance in both clinical diagnosis and the development of novel treatment strategies aimed at mitigating cardiovascular risk.

MATERIALS AND METHODS

180 blood sample was collected from Between January and December 2024, from people aged 35 to 75 years old, they were examined at Al Salam Hospital -Cardiac Surgery Center- Mosul. We assort the samples into two groups: first group included 90 patients diagnosed with atherosclerosis second group comprised 90 control samples. Then Sixty blood sample were collected for molecular study, these samples assorted into two groups: first group included 40 patients diagnosed with atherosclerosis second group comprised 20 control samples

2.1 BLOOD SAMPLE COLLECTION:

five milliliters of venous blood was withdrawn from each person then divided into groups. The first group was placed in gel tubes, then tubes were placed in a centrifuge at 3000 rpm for 10 minutes. The serum was withdrawn from the other components of blood and transferred to 2 ml Eppendorf tubes. second group was kept in a tube with EDTA for DNA extraction, while the third group was left to be placed in a tube with Triazole for RNA extraction [9].

2.2 ESTIMATION OF METEORIN-LIKE PROTEIN LEVEL IN HUMAN BLOOD SAMPLE:

The Sandwich kit with Cat. No E3941Hu from BT LAB Chines Company was used to detect Human Meteorin-Like Protein in serum of blood sample accurately and quantitatively; this kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate was pre-coated with Human Meteorin-Like Protein antibody, Meteorin -like protein present in the blood serum sample was added to bind to antibodies coated on the plate wells, then biotinylated Human Meteorin-Like Protein antibody is added and binds to Meteorin Like Protein in the sample. After that Streptavidin-HRP was added and binds to biotinylated Human Meteorin-Like Protein Antibody, then incubated and washed to remove unbound Streptavidin-HRP and a substrate solution is added, the developed color is proportion to the amount of Human Meteorin-Like Protein in the sample, finally the rection was terminated by the way of adding stop acidic solution and the absorbance was measured at 450 nm.

The q-PCR technique was conducted to assess the level of gene expression for Metrnl genes, and the procedure involved many stages.

2.3 RNA EXTRACTION: After collecting blood from the participants, 750 µL of Triazole was mixed quickly and efficiently by the aid of a vortex mixer, with 250 µL of blood, for both patient and control samples. RNA was extracted by applying the special protocol and instructions that was provided with TransgenBiotech kit, while its purity was assessed by using a NanoDrop device [10].

2.4 CONVERTING EXTRACTED mRNA to cDNA: The extracted RNA was transformed into cDNA upon the dependence on the activity of the reverse transcriptase enzyme by using TransgenBiotech kit and application of the protocol that was provided with the kit.

The levels of gene expression were quantified by using specific primers for the Metrnl gene and a housekeeping gene, as detailed in Table 1.

The qPCR reaction was conducted in a full volume of 20 µl, using Ultra SYBR Green q-PCR master mix, forward primer, reverse primer as well as cDNA template, and distilled water. While the qPCR program involve an initial pre-denaturation step at 95°C for 10 minutes, succeeded by denaturation at 95°C for 15 seconds, as well as annealing/extension at 60°C for 1 minute, and finally a melting curve analysis was used at multiple temperature stages [11].

Table 1: METRNL and House Keeping gene Primers utilized in the RT-PCR process

Primer	Sequence
METRNL F	CTGCCTCTCCAAGAGTGTAGC
METRNL R	CACTGCCGACGGTATTTGTA
H.K-F	GACCCAGATCATGTTTGAG
H.K-R	GACCCAGATCATGTTTGAG

CALCULATING OF GENE EXPRESSION FOLDING: (LEVAK CALCULATE)
In this research, folding of gene expression was calculated by using the double ΔCT method (ΔΔCT) based on the following equation: Gene Expression folding = 2 ΔΔCT[12].

2.6 DNA EXTRACTION
DNAwas extracted by applying the special protocol and instructions that was provided with TransgenBiotech, in which Proteinase K solution, Lysis buffer-2, Washing buffer 1, Washing buffer 2, Elution buffer as well as Collection tube and spin column were used then Biodrop spectrophotometer was used to determine both concentration and purity of the genomic DNA that was extracted from the samples

2.7 DNA SEQUENCE ANALYSIS
In this study, the sequences of the nitrogenous bases of the METRNL gene were determined to make sure the accuracy of the designed primer used in the PCR technique also to identify the presence of the genetic variation in the target genes that may influence the effect on the gene effectiveness as well as its rule in the development of the disease. The products of the PCR reaction for the Metrnl genes were sent with the primers. Then the sequence was read at the Psomagen Center in the United States of America. After that, the obtained gene sequences were matched with the reference gene sequences documented at the National Center for Biotechnology Information (NCBI). Finally, the results were analyzed by using the bioinformatics Basic Local Alignment Search Tool (BLAST) program. The sequences of primers used are shown in table (2)

PCR reaction was conducted, using the DNA, For. Primer, Rev. primer, Master mix of PCR and Distilled water. While the qPCR program involve an 1 cycle initial pre-denaturation step at 94°C for 5 minutes, succeeded by 35 cycle denaturation at 94°C for 45 min, as well as 1 cycle of annealing at 57OC for 1 minute as well as 1 cycle of extension at 72°C for 1 minutes followed by 1 cycle of final extension at 72 o C for about 1 minute and finally 1 cycle of stop reaction was used at 4o C for 4 minutes.

Primer	Sequence
METRNL F	CTGCCCCGTGAAGGTCAG
METRNL R	CTTCTCGGAAAACAGCTTCG

Table 2: displays primer sequence used in the DNA sequenc

2.8 STATISTICAL ANALYSIS

Analysis of the results was done by using the statistical program SPSS 25, An independent sample t-test is used to compare the means of healthy and patient groups to determine if there is a statistically significant difference between them while the receiver operating characteristic (ROC) curve analysis was utilized to assess the diagnostic performance of Meteorin-Like Protein and area under the curve (AUC). [13].

RESULTS

A total 240 blood sample was collected from individuals, including patients diagnosed with atherosclerosis and healthy controls ,180 of them for biochemical study and 60 blood sample were collected for molecular biology study.

3.1 THE LEVEL OF METEORIN-LIKE PROTEIN IN SERUM OF CONTROL AND PATIENTS WITH ATHEROSCLEROSIS

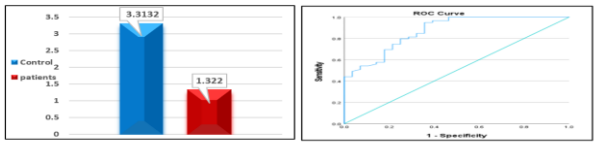

Fig. 1: a. Comparison of Meteorin-Like Protein levels between the healthy control group and patients at P ≤0.001 b.Diagnostic accuracy of serum Metrnl level in the predication atherosclerosis evaluated by ROC curve

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Table 3: Analysis of ROC Curve of Meteorin-Like Protein in Atherosclerosis Predicting.

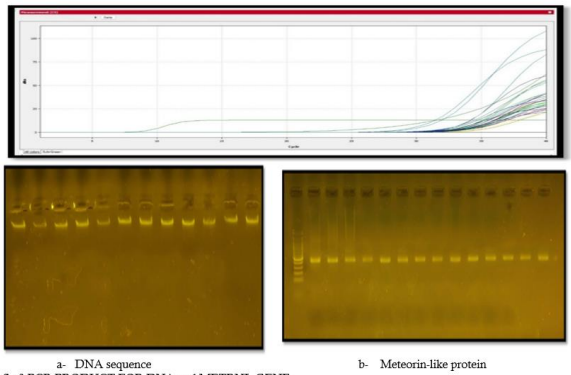
Cut-off	AUC	Sensitivity	Specificity	Std.Errora	Asymptotic Sig.b	Asymptotic 95% Confidence Interval	
						Lower Bound	Upper Bound
≤ 2.2585	0.871	0.949	0.643	0.040	0.000	0.792	0.950

MEASURING THE LEVEL OF GENE EXPRESSION OF THE METRNL AND ANALYSIS OF DNA SEQUENCE RESULTS.

Table (4): gene expression level of metrnl and the housekeeping gene in control group and atherosclerosis patients in the case study

Sample	CT target gene	CT housekeeping gene	Δ CT target gene	ΔCT control	Δ Δ CT	Gene Expression folding
Control	37.06	38.8	-1.74	-1.74	0	1
Patients	37.337	37.735	-0.116	-1.74	1.618	0.451

Fig.2: melting curve for q PCR and the level of atherosclerosis Metrnl gene measured in this study.



a- DNA sequence b- Meteorin-like protein

fig.3 PCR PRODUCT FOR DNA and METRNL GENE

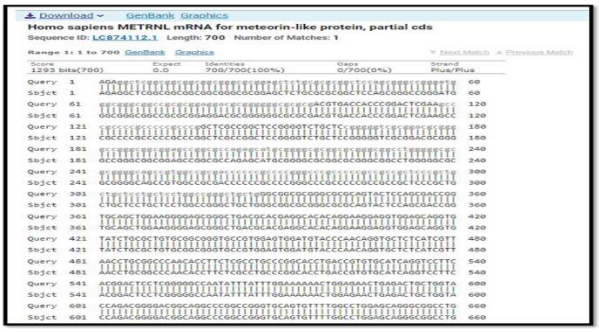


Fig.4:the matching results of nucleotide sequence of the Metrnl gene in the studied blood sample with the nucleotide sequence of the main gene at national center of biotechnology information (NCBI)

DISCUSSION

Arteries are essential conduits of the circulatory system, along with veins and the heart. They are tube-like structures responsible for transporting fluids—namely blood in the circulatory system and lymph in the lymphatic system—to and from every organ in the body. Fundamentally, arteries transport oxygenated blood and nutrients to body tissues[14] Atherosclerosis is a multifocal inflammatory disease of the arterial vessels and represents the principal underlying cause of heart disease and stroke [15]. The condition often originates in adolescence and gradually advances with age. It is marked by lipid accumulation, inflammatory cell infiltration, smooth muscle proliferation, and extracellular matrix deposition within the intimal layer [16]. Atherosclerotic cardiovascular disease (ASCVD) develops through the interaction of genetic predisposition with multiple modifiable along with non-modifiable risk factors.

Recent research highlights the involvement of epigenetic regulation in disease progression. Meteorin-like protein (Metrnl) has recently attracted attention for its potential role in vascular pathology. This study was designed to assess its involvement in atherosclerosis.

Results shows that the level of Meteorin-Like Protein is Serum of atherosclerosis patients is significantly decreased (mean = 1.322ng/ml) as compared to the healthy control group (mean = 3.3132 ng/ml) as shown in Figure1: (a). This notable reduction suggests that there is an inverse relationship between Meteorin-Like Protein levels and atherosclerosis disease. ROC curve analysis Figure1: (b) & Table (3) establish a very good diagnostic accuracy, which reveal area under the curve (AUC) of 0.871 for serum Meteorin-Like Protein level. The best cut-off value of serum its level that predicted atherosclerosis was ≤2.2585 ng/ml, and the sensitivity and specificity were 0.949 and0.643 respectively These results indicate that serum meteorin-like protein has reasonable characteristic power and could serve as a biomarker for prediction of atherosclerosis.

The qPCR amplification curves demonstrated clear differences in Metrnl gene expression between patients with atherosclerosis and healthy controls. As shown in table (4) and the amplification plot figure (2), patient samples exhibited delay threshold cycle (Ct) values compared to controls, indicating a reduced abundance of Metrnl transcripts. This observation was further confirmed by the relative quantification analysis, where the normalized expression level of Metrnl in the patient group (0.45) was markedly lower than that of the control group (1.0). These results indicate a 55% decrease in Metrnl expression among those with atherosclerosis.

These findings suggest that Metrnl expression is significantly downregulated in individuals with atherosclerosis. The observed reduction may reflect its involvement in vascular homeostasis and inflammation, processes that are dysregulated during the development of atherosclerotic lesions. Given its consistent decrease in patients, Metrnl could potentially serve as a biomarker for disease progression and may represent a novel therapeutic target. Nevertheless, further studies with larger cohorts and mechanistic investigations are required to clarify its precise role in the pathophysiology of atherosclerosis

The downregulation of Metrnl observed in atherosclerosis patients aligns with previous studies suggesting a disease suppressive role. For example, research conducted by Huang& Hou in 2023 found that lower Metrnl levels were linked with a higher risk of both first-time and recurrent ACS. Even after adjusting for the usual risk factors (like high cholesterol, diabetes, hypertension, smoking) so Metrnl could be a protective factor for the heart[17]. Another research found that in a mouse model of doxorubicin-induced cardiotoxicity, METRNL activates the cAMP/PKA/SIRT1 signaling pathway, conferring resistance to myocardial oxidative stress, apoptosis, and myocardial injury[18] . Future studies should seek to investigate the mechanistic contributions of Metrnl to atherosclerosis progression, consider its potential rule as a biomarker for early

detection of atherosclerosis, and to assess its therapeutic applications.

Analyses Of Sequence Results

From the results of the DNA sequence analysis and when the results of nucleotide sequence of the Metrnl gene with in the studied blood sample matched with the nucleotide sequence of the main gene at national center of biotechnology information (NCBI) as shown in figure(3 and 4), this BLAST result show that the Metrnl DNA sequence which successfully deposited in the GenBank (Accession number: LC874112 (is exactly identical (100% match) to Metrnl sequence in the database nucleotide sequence of the main gene at national center of biotechnology information (NCBI), this matching reflect that there is no mutation within the gene sequence and the disease progression may result from unhealthy lifestyle, dietary habits , environmental effects and obesity.

CONCLUSION

Metrnl rule as atherosclerosis disease progression suppressor, indicated by its significant downregulation in atherosclerosis patients. This notable reduction in expression could influence the severity or progression of the disease. disease progression may result from unhealthy lifestyle, dietary habits , environmental effects and obesity These findings suggest that Metrnl holds potential as a biomarker for diagnosing of atherosclerosis and its complications and may serve as a treatment target.

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ETHICAL CLEARANCE: ethics committee of Nineveh health directorate (department of health, Mosul, Iraq) approved the study under official documentNo.59239, date11/12/2024.

CONFLICT OF INTEREST. No conflict

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