Palestinian Medical and Pharmaceutical Journal

Metrnl: A predictive marker of atherosclerosis among Rheumatoid Arthritis patients

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Received 4th Sep 2024, Accepted 21th Feb 2025, Published:×××× , DOI: https://doi.org/10.xxxx

Accepted Manuscript, In press

Abstract: Metrnl is a novel secreted protein exerting pleiotropic effects on inflammation and metabolism. Metrnl is increased in rheumatoid arthritis patients, however, Metrnl deficiency causes endothelial dysfunction which in turn promotes atherosclerosis. This study aimed to analyze serum Metrnl in Rheumatoid Arthritis patients with and without atherosclerosis. A case-control study was conducted on 50 Rheumatoid patients compared with 50 sex and age-matched control group. Metrnl levels were measured in blood samples from patients and controls by Enzyme-linked immunosorbent assay (ELISA), and CIMT was measured using Doppler ultrasonography. Medical data were collected regarding the patients' clinical manifestations, comorbidities, and treatments. The disease activity score-28 (DAS28) was used to evaluate the disease activity of RA. Metrnl, right, and left CIMT showed a significant increase in the RA group when compared to the control group. Metrnl level showed a significant negative correlation with CRP, cholesterol, right CIMT, and left CIMT. However, no significant correlation was found between Metrnl and other parameters. At a cut-off point, ≥1.0748 Metrnl level showed significant AUC with 65% specificity and 76% sensitivity. At cut-off point ≥ 0.55, right and left CIMT levels showed significant AUC with 82% specificity and 48% sensitivity for differentiation between cases and controls. Age, BMI, disease duration, Family history of premature atherosclerosis, CRP, ESR, TG, cholesterol level, LDL, HDL, and lower Metrnl were associated with increasing CIMT in rheumatoid patients in univariate analysis. However, in multivariate analysis, only age, ESR, TG, HDL, and Metrnl were associated with increasing CIMT in rheumatoid patients. **In Conclusion:** Metrnl increased in patients with RA while lower Metrnl levels were correlated with increased CIMT in cases. Age, BMI, disease duration, Family history of premature atherosclerosis, CRP, ESR, TG, cholesterol level, and LDL, were linked to an increase in CIMT in rheumatoid patients

Keywords: Atherosclerosis, Carotid intima-media thickness, Metrnl, Rheumatoid arthritis

Introduction:

Rheumatoid arthritis (RA) is a chronic, inflammatory joint disease that can lead to joint destruction. RA particularly attacks peripheral joints, gradually resulting in bone erosion, destruction of cartilage, and, ultimately, loss of joint integrity. The prevalence of RA varies globally, ranging from 0.1- 2.0% of the population worldwide, and significantly reduces functional capacity and quality of life [1]. Any joint lined by a synovial membrane may be involved, but extra-articular involvement of organs such as the skin, heart, lungs, and eyes can be significant. RA is theorized to develop when a genetically susceptible individual experiences an external trigger (e.g., cigarette smoking, infection, or trauma) that triggers an autoimmune injury [2].

RA patients have an increased risk of Cardiovascular (CV) related morbidity and mortality. Both traditional CV risk factors and RA-specific features contribute to excess CV death. Hence, traditional CV risk-assessing tools used in the general population largely disappoint in RA. RA is an independent risk factor for CVD and close association with disease activity has been shown in multiple studies [3]. Genetic factors and the influence of several gene polymorphisms in the risk of accelerated atherosclerosis of patients with RA were highlighted in the research [3].

Cardiovascular risk factors were independently associated with increasing common carotid artery intima-media thickness, plaque prevalence, and plaque scores [4]. Ultrasound measurement of carotid intima-media thickness (CIMT) and plaque thickness (PT) may be an additional tool for risk stratification of patients with atherosclerosis and subclinical coronary syndrome [5].

Meteorin-like (Metrnl) is a Novel cytokine encoded by a gene called Metrnl gene, which is a small (~28 kDa) secreted protein expressed by active macrophages and barrier tissues (mucosa and skin). Metrnl production by bone marrow macrophages is induced by several cytokines including TNF-a, IL-17a, IL-12, and IL-4, and inhibited by IFN-g and TGF-b. Metrnl functions include important regulatory roles in metabolism and inflammation and in both innate and acquired immunity, metrnl is abundantly expressed in

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metabolism-related organs and barrier tissues. Most clinical studies focus on the relationship between metrnl and metabolic disorders such as type 2 diabetes, coronary heart disease, or inflammatory diseases such as colitis, and arthritis [6, 7]. Metrnl function as an endothelium-derived factor that help maintaining endothelial functions against atherosclerosis, an adipokine for insulin sensitization, a cardiokine against cardiac dysfunction, Recent findings give a key explanation for Metrnl functions in circulation and its role in cardiovascular health and disease, these clinical studies help understand the clinical effects of metrnl and demonstrate its potential as a new therapeutic target [8].

This study aimed to analyze the correlation between serum Metrnl and carotid intimal medial thickness (as a marker of atherosclerosis) in Rheumatoid Arthritis patients, to the best of our knowledge, this is the first study that analyzed Metrnl relation to rheumatoid and atherosclerosis at the same time.

Patients and methods:

The present study was a case control study conducted on RA patients attending the rheumatology and immunology outpatient clinics and inpatient wards, at Menoufia University Hospital. The study was approved by the ethical committee of Menoufia University with code 3/2022INTIM42, and it was carried out according to the scientific research ethics guidelines. Before the initiation of the study, each subject was informed about the aim of the study and signed an informed consent form.

The present study was conducted on two groups; Group I included 50 Rheumatoid adult patients and Group II included age & sex-matched (40) control subjects. Sample size was calculated in the public health and community medicine department based on the review of past literature Gholamrezaya et al **[9]**. the least sample size calculated using G POWER program version 3 in 80 participants divided into two groups. The power of the study is 80% and confidence level is 95%.

Patients with RA were diagnosed according to the 2010 ACR / EULAR Rheumatoid Arthritis Classification Criteria [10]. Patients excluded from the study include those with any other connective tissue disease as systemic lupus erythematosus, scleroderma, osteoarthritis, or antiphospholipid syndrome, also patients using lipid-lowering drugs and adult females more than 65 years and less than 18 years, smokers were excluded from our study. All the participants were asked about their medical history and underwent a complete physical examination.

A venous blood sample (3 ml) was withdrawn from each participant in the plain tube and then centrifuged to get a serum sample for laboratory chemical investigations. Routine investigations included CBC, blood film, Serum Lipid profile (Total cholesterol, Triglycerides (TG), HDL-Cholesterol, and LDL-cholesterol), fasting and 2 hr. postprandial blood glucose, HbA1C, Rheumatoid Factor (RF), Anti-Cyclic citrullinated peptide (Anti-CCP), Antinuclear Antibody (ANA), Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), serum ferritin, liver and kidney functions were conducted for all enrolled subjects by appropriate chemical principles. Serum Meteorin-like (Metrnl) was assayed by ELISA kits supplied by SunRed (Catalogue No. SRB-T-81862).

The distance between the leading margins of the first and second echogenic lines on both sides of the far walls of the common carotid artery's distal segment and its bifurcation and internal carotid artery was measured using Doppler ultrasonography (GE logic E10 USA) and a duplex ultrasound system operating in B-mode at 7.5 MHz scanning frequency. Measurements were conducted at intervals of 0.5, 1, and 2 cm below and above the bifurcation point, respectively. It took an average of three measures to come up with each result, and just one person was involved in the measuring process. The statistical comparison of CIMT thickness between patients and control groups was carried out using the mean values.

Statistical analysis

Data were collected, tabulated, and statistically analyzed using an IBM-compatible personal computer with Statistical Package for the Social Sciences version 26 (SPSS Inc. Released 2015. IBM SPSS Statistics for Windows, version 26.0, Armnok, NY: IBM Corp.). Qualitative data were expressed as Number (N), and percentage (%), while quantitative data were expressed as mean ($\bar{x}$), standard deviation (SD) or median (IQR). Shapiro-Wilk and Kolmogorov-Smirnov tests are used to assess the normality of distribution. (Snecdecor GW, Cochran WG. Statistical Methods: Wiley; 1991. 14-29 p). Student's t-test (t) was used for the comparison of quantitative variables between two groups of normally distributed data, while Mann-Whitney's test (U) was used for the comparison of quantitative variables between two groups of not normally distributed data. (Hollander M, Wolfe DA, Chicken E. Nonparametric statistical methods. John Wiley & Sons; 2013 Nov 25). The chi-square test was used to study the association between qualitative variables was used. (Powers D, Xie Y. Statistical methods for categorical data analysis. Emerald Group Publishing; 2008 Nov 13). Whenever any of the expected cells were less than five, Fischer's exact test was used. Spearman rank correlation coefficient was used to correlate 2 nonparametric variables. Receiver Operator Characteristic (ROC) curves with the Area under the Curve (AUC) were used to determine the optimal cut-off. To investigate risk factors for increased CIMT, the univariate and multivariate logistic regression analyses were performed. Factors with p value <0.05 in univariate analysis were included in multivariate analysis. The results were expressed as adjusted odds ratio (OR) with 95% confidence interval (CI).

Results:

The mean age of the studied cases was 46.18±10.39 and most of them were female 98%, most cases had no comorbidities, and the most common associated comorbidity was diabetes, all cases had positive RF/ Anti CCP. Median CRP was 18.5, ESR was 38, TG 94, Cholesterol was 152, LDL 99.5, HDL 57.5, Metrnl was1.27, Right CIMT was 0.7, Left CIMT was 0.71 **(Table 1).**

Family history, CRP, ESR, TG, cholesterol, LDL, Metrnl, and right and left CIMT showed an increase in the cases group when compared to the control group. No significant difference was found between the studied groups regarding age, sex, BMI, comorbidities, and HDL (P>0.05) **(Table 2).**

Right CIMT and Left CIMT showed a positive correlation with age, disease duration, CRP, TG, cholesterol, and LDL. Also, they showed a negative correlation with Metrnl **(Table 3).**

Metrnl level showed a significant negative correlation with CRP, cholesterol, right CIMT, and left CIMT. However, no significant correlation was found between Metrnl and other parameters (**Table 4)**

At a cut-off point ≥1.0748, Metrnl level showed significant AUC with 65% specificity and 76% sensitivity. At a cut-off point ≥ 0.55 right and left CIMT levels showed significant AUC with 82% specificity and 48% sensitivity **(table 5 and Figure 1).**

Metrnl level ≤1.26 showed significant AUC with a sensitivity of 60% and specificity of 72% in the determination of high CIMT **(table 6).**

Table (1): Demographic, clinical, and laboratory characteristics of studied patients (N=50):

Variable	Studied cases (N=50)
	No. (%)
Age (years): (Mean ±SD)	46.18±10.39
Sex Male Female	1(2.0) 49(98.0)
BMI (Mean ± SD)	30.52±3.76
Family history of premature atherosclerosis yes	7(14.0)
Comorbidities None Diabetes DM&HTN CVS	31(62.0) 8(16.0) 4(8.0) 7(14.0)
Disease duration (Mean ± SD) range	7.42±5.28 1-28
DAS (Mean ± SD) range	3.72 ±0.97 2.4-7.2
Extra-articular manifestations Positive Negative	10(20.0) 44(80.0)
RF/ Anti CCP Positive	50(100.0)
CRP Median (IQR)	18.5(12 – 30.75)
ESR Median (IQR)	38.5(28.75 – 50)
TG Median (IQR)	94 (85.75 – 110.75)
Cholesterol Median (IQR)	152(115–186.5)
LDL Median (IQR)	99.5(88.75 –119.5)
HDL Median (IQR)	57.5(40.75 -68)
Metrnl Median (IQR)	1.27 (1.064 – 1.593)
Right CIMT Median (IQR)	0.7(0.6 -0.8)
Left CIMT Median (IQR)	0.71(0.6 -0.8)

SD: standard deviation, range: minimum maximum, No: number, %: percentage, IQR=inter quartile range (25-75) **Anti CCP** anti cyclic citrullinated peptide**s, BMI**; body mass index **CIMT** carotid intima-media thickness, **CRP**; C - reactive protein, **DM** diabetes mellitus **DAS**: Disease Activity Score, **ESR**; erythrocyte sedimentation rate, **HTN** hypertension, **RF** Rheumatoid factor, **TG** triglyceride, **HDL**; high-density lipoprotein, **LDL**; low-density lipoprotein

Age, BMI, disease duration, Family history of premature atherosclerosis, CRP, ESR, TG, cholesterol level, LDL, HDL, and Metrnl were associated with increasing CIMT in rheumatoid patients in univariate analysis. However, only age, ESR, TG, HDL, and Metrnl were associated with increasing CIMT in rheumatoid patients in multivariate analysis **(Table 7).**

Table (2): comparison between patients and controls regarding demographic, clinical, and laboratory markers:

Variable	Cases (No.=50) No. (%)	Controls (No.=40) No. (%)	Test of significance	P value
Age (years): (Mean ±SD)	46.18±10.39	42.13±12.68	t=1.667	0.099
Sex Male Female	1(2.0) 49(98.0)	4(10.0) 36(90.0)	FE =2.711	0.167
BMI (Mean ± SD)	30.52±3.76	29.2±3.79	t=1.652	0.102
Family history yes	7(14.0)	0(0.0)	FE =6.072	0.019*
Comorbidities None Diabetes DM&HTN CVS	31(62.0) 8(16.0) 4(8.0) 7(14.0)	29(72.5) 5(12.5) 5(12.5) 1(2.5)	$\chi 2$ =4.312	0.230
CRP Median (IQR)	18.5(12 – 30.75)	2(2 – 10)	U=7.933	<0.001**
ESR Median (IQR)	38.5(28.75 – 50)	15(9.25 – 17.75)	U=8.099	<0.001**
TG Median (IQR)	94 (85.75 – 110.75)	55(42 -59)	U=8.131	<0.001**
Cholesterol Median (IQR)	152(115–186.5)	95(87– 98)	U=8.125	<0.001**
LDL Median (IQR)	99.5(88.75 –119.5)	75(65 - 77.9)	U=8.107	<0.001**
HDL Median (IQR)	57.5(40.75 -68)	81 (77.75– 84.75)	U=8.125	<0.001**
Metrnl Median (IQR)	1.27 (1.064 – 1.593)	1.04 (0.864– 1.39)	U=2.96	0.003*
Right CIMT Median (IQR)	0.7(0.6 - 0.8)	0.5 (0.425 -0.5)	U=8.117	<0.001**
Left CIMT Median (IQR)	0.71(0.6 - 0.8)	0.5(0.5 - 0.51)	U=8.233	<0.001**

SD: standard deviation, range: minimum maximum, No: number, %: percentage *P value of < 0.05: statistically significant., **P value of < 0.001: statistically highly significant. U= Mann-Whitney , $\chi 2$ =Chi square , t = student t test FE=Fisher's Exact Test **BMI**; body mass index **CIMT** carotid intima-media thickness, **CRP**; C - reactive protein, **CVS** cardiovascular disease , **DAS**: Disease Activity Score **DM** diabetes, HTN hypertension, **ESR**; erythrocyte sedimentation rate, , **HDL**; high-density lipoprotein, **LDL**; low-density lipoprotein , **TG** triglycerides.

Table (3): Correlation between carotid artery intima-media thickness & other variables of the studied patients (n=50)

Variables	Right CIMT		Left CIMT	
	Rho	P value	Rho	P value
Age (years):	0.792	<0.001**	0.807	<0.001**
BMI	0.302	0.033*	0.313	0.027*
Disease duration	0.822	<0.001**	0.813	<0.001**
DAS	-0.029	0.84	-0.042	0.774
ESR	0.911	<0.001**	0.921	<0.001**
CRP	0.872	<0.001**	0.879	<0.001**
TG	0.851	<0.001**	0.868	<0.001**
Cholesterol	0.916	<0.001**	0.919	<0.001**
LDL	0.939	<0.001**	0.934	<0.001**
HDL	-0.878	<0.001**	-0.895	<0.001**
Metrnl	-0.320	0.023*	-0.302	0.033*

P value of < 0.05: statistically highly significant *P value of < 0.05: statistically significant. **rho =spearman correlation coefficient **BMI**; body mass index **CIMT** carotid intima-media thickness, **DAS**: Disease Activity Score, CRP; C- reactive protein, **ESR**; erythrocyte sedimentation rate, **HDL**; high-density lipoprotein, **LDL**; low-density lipoprotein, **TG;** triglycerides.

Table (4): Correlation between Metrnl level & other variables of the patients (n=50)

Variables	Metrnl level	
	Rho	P value
Age (years):	-0.224	0.117
BMI	0.159	0.270
Disease duration	0.211	0.141
DAS	0.027	0.850
ESR	-0.252	0.077
CRP	-0.327	0.02*
TG	-0.190	0.187
Cholesterol	-0.328	0.02*
LDL	-0.242	0.09
HDL	0.209	0.145
Right CIMT	-0.320	0.023*
Left CIMT	-0.303	0.033*

*P value of < 0.05: statistically significant **rho** =spearman correlation coefficient

BMI; body mass index **CIMT** carotid intima-media thickness, **DAS**: Disease Activity Score **CRP**; C - reactive protein, **ESR**; erythrocyte sedimentation rate, , **HDL**; high-density lipoprotein, **LDL**; low-density lipoprotein, **TG** triglycerides.

Table (5): Receiver operating characteristic curve analysis of the optimal cutoff of CIMT and Metrnl levels

Cutoff point	AUC	Sensitivity%	Specificity%	P value	95% CI Lower - Upper
Metrnl level					
≥1.0748	0.682	76%	65%	0.003*	0.570 – 794
Right CIMT					
≥ 0.55	0.985	96%	100%	<0.001**	0.961 – 1
Left CIMT					
≥0.55	0.992	98%	100%	<0.001**	0.975 – 1

P value of < 0.001: statistically highly significant - **CIMT carotid intima-media thickness,

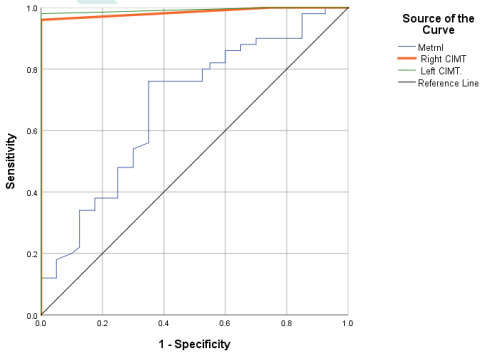

Figure (1): Receiver operating characteristic curve analysis of the optimal cutoff of carotid intima-media thickness and Metrnl levels

Table 6: Receiver operating characteristic curve analysis of the optimal cutoff of Metrnl levels to determine high carotid intima-media thickness:

Cutoff point	AUC	Sensitivity%	Specificity%	P value	95%CI Lower – Upper
Metrnl level					
≤1.26	0.706	60%	72%	0.014*	0.564 – 849

Table (7): **Univariate and Multivariate analysis for variables significantly associated with increased CIMT in rheumatoid patients.**

Predictors (Independent variables)	Univariate regression				multivariate regression			
	B coefficient	Odds Ratio (OR)	P value	95% CI (lower-upper)	B coefficient	Odds Ratio (OR)	P value	95% CI (lower-upper)
Age (years): (≥46 years)	3.604	36.750	<0.001**	6.642–203.351	2.697	14.836	0.012*	
Sex (female)	-21.203	0	1	0-0				
BMI (≥30.5)	0.183	1.201	0.035*	1.013 -.423	0.201	1.222	0.123	1.222 – 1.578
Duration of disease (≥7years)	2.811	16.625	<0.001**	4.062 - 68.038	1.650	5.209	0.072	0.865 – 31.378
Family history of premature atherosclerosis (yes)	2.416	11.2	0.032*	1.231 – 101.886	2.554	1.078	0.147	0.002 – 2.454
DAS (≥3.72)	0.302	1.352	0.669	0.340 – 5.386				
Extra articular (Positive)	1.466	4.333	0.055	0.966 -19.43				
CRP (≥18.5)	2.576	13.143	<0.001**	3.292 - 52.466	1.780	5.930	0.092	1.747 – 47.093
ESR (≥38.5)	3.030	20.7	<0.001**	4.845–88.44	2.969	19.47	0.048*	1.028 – 368.582
TG (≥94)	2.423	11.281	<0.001**	2.9 – 43.878	2.657	14.248	0.034*	1.219 - 166.57
Cholesterol (≥152)	2.791	16.292	<0.001**	3.970 – 66.9	1.622	50.064	0.098	1.740-36.653
LDL (≥99.5)	2.534	12.6	<0.001**	3.288–48.287	0.193	1.213	0.902	0.56 – 26.519
HDL (≥57.5)	-2.811	0.06	<0.001**	0.015 – 0.246	-3.154	0.043	0.026*	0.003 – 0.681
Metrnl (<1.27)	1.781	5.937	<0.008*	11.211 - 335.5	2.676	14.528	0.041*	1.120 – 188.42

CI= Confidence interval *P vale of < 0.05: statistically significant. **P value of < 0.05: statistically highly significant. **BMI**; body mass index **CIMT** carotid intima-media thickness, **DAS**: Disease Activity Score **CRP**; C - reactive protein, **ESR**; erythrocyte sedimentation rate, **TG** triglyceride, **HDL**; high-density lipoprotein, **LDL**; low-density lipoprotein

Discussion:

Rheumatoid arthritis is an independent risk factor for cardiovascular disease (CVD). The causal relationship between RA and CVD has remained unclear. Although patients with RA were not at a higher risk of hyperlipidemia than the general population, a higher risk of CV events was found in patients with RA compared with individuals without RA **[11 ,12].**

The present study showed that Metrnl showed a significant increase in the RA group when compared to the control group. The cut-off point ≥1.0748 Metrnl level showed significant AUC with 65% specificity and 76% sensitivity for differentiation between RA cases and controls. Moreover, we found that Metrnl level showed a negative correlation with CRP, cholesterol, right CIMT, and left CIMT. However, no significant correlation was found between Metrnl and other studied parameters.

In the present study A cut-off value of Metrnl level ≤1.26 showed significant AUC , rendered the sensitivity of 60% and specificity of 72% respectively, in the determination of high CIMT, Furthermore, an attempt was made to define the appropriate "cut-off" value for defining atherosclerosis , a cut-off value of CIMT among the normal control subjects was also used as the optimal cut-off value for defining the presence of atherosclerosis in patients with RA. The proportion of patients with atherosclerosis among rheumatoid cases and controls detected using cut-off values of metrnl and CIMT as surrogate markers. CIMT measurements obtained with doppler ultrasonography were used to construct an ROC curve and a cut-off value of 0.55 was derived, Patients with RA were categorized as having atherosclerosis if their CIMT value was greater than or equal to 0.55 mm. The performance of this cut-off value in detecting asymptomatic atherosclerosis among patients with RA was similar to the 75th percentile value of CIMT in normal control subjects, Thus, appeared to be appropriate for screening for subclinical athero-sclerosis among RA patients.

This was in line previous study by Gonzalez-Gay et al who observed that Long-standing RA patients with mean C-reactive protein (CRP) levels greater than 15 mg/dl had higher CIMT values than those with lower CRP levels **[13].** Also, Tutoğlu et al observed a positive correlation between the mean CIMT score and age, CRP levels, LDL concentration, and triglycerides (TG) level **[14].**

Previous studies found circulation Metrnl levels were reduced in atherosclerosis and coronary artery disease, patients and negatively correlated with endothelial parameters, moreover, circulating Metrnl was lower in elderly patients with chronic heart failure and negatively correlated with cardiovascular mortality, frequent hospitalization, and multiple adverse cardiac **[15, 16]**. Of note, a previous study has demonstrated that Metrnl could ameliorate lipopolysaccharide induced endothelial cells' inflammatory response **[17]**. CIMT is a predictor of atherosclerosis and impending cardiovascular events, indicating metrnl

involvement in atherosclerosis. Endothelial impairment factors could facilitate Metrnl secretions by and circulation Metrnl levels decreased significantly with the Atherosclerosis progression. Moreover, areas of aortic plaques, necrotic injuries, and lipid accumulations of aortic root were heavier in aorta plaque of endothelial Metrnl deficiency mice models than those in control, suggested that the Atherosclerosis pathogenesis is related to Metrnl deficiency in endothelial cells **[18].**

C-reactive protein elevation correlates with accelerated atherogenesis in patients with rheumatoid arthritis, whereas long-standing RA patients with mean C-reactive protein levels greater than 15 mg/dl had higher CIMT values than those with lower CRP level **[19].** previous studies observed that serum metrnl concentrations are negatively correlated with CIM. The effects on both adiposity and inflammation could explain the inverse association of Metrnl expression with CIMT in our study **[20].** A previous case-control study included sera from 159 RA patients, 28 osteoarthritis (OA) patients showed that Metrnl is involved in the pathogenesis of RA. An increase in serum Metrnl levels is closely related to RA activity (**21).**

The mean age of the studied cases was 46.18±10.39 and most of them were female 98%. The female predominance in the present study agreed with Garrigues et al Found that 29 (72%) were female, the mean age in their study was 55.9 years (SD: 14) and the mean disease duration was 11.2 years (SD: 8.7) **[22].** Many factors have been implicated in the overrepresentation of women in RA, including hormonal, genetic, lifestyle, and environmental factors **[23].**

The present study showed that most cases had no comorbidities, and the most associated comorbidity was diabetes followed by cardiovascular disease. Previous studies demonstrated that the most common comorbidities in patients with RA are hypertension and osteoporosis or osteopenia **[24].**

The present study showed that all cases had positive RF/ Anti CCP. In agreement with our results [25] found that RF was positive in 80% of patients. Several case-control studies on this issue in RA patients found that anti-CCP was positive in 50% of cases. It was found that rheumatoid factor (RF) was positive in 30 (71.4%) patients and negative in 12 (28.6%) patients. The mean ± SD positive RF value was 135 ± 160 IU/ml (range 16–880 IU/ml) [26]. In another study of twenty-five patients known patients with rheumatoid arthritis, 19 (76%) patients showed elevated ACCP & RF. Rheumatoid factor was positive in 52% of RA patients. There is a female predominance, younger age, and more prevalent polyarticular involvement at disease onset, lower extra-articular manifestations, and less erosive disease in Egyptian RA patients [27].

On lipid profile, we found that TG, cholesterol, and LDL showed a significant increase in the cases group when compared to the control group. No significant difference was found between the studied groups about BMI, comorbidities, and HDL. Carotid ultrasound measures carotid intima-media thickness (CIMT) to assess cardiovascular disease in diabetic patients. Brachial-ankle pulse wave velocity is a well-known index of arterial stiffness and can predict mortality in diabetic patients. Atherogenic indices such as LDL/high-density lipoprotein (HDL) ratio, atherogenic index, and CIMT were higher in RA patients **[28].** METRNL deficiency causes endothelial dysfunction which in turn promotes the susceptibility of atherosclerosis **[29, 30].**

clinicians should routinely assess traditional cardiovascular risk factors such as lipid profiles, blood pressure, and body mass index. Comprehensive cardiovascular risk assessment and management should be integrated into the overall care plan for RA patients. Further research is warranted to explore the potential role of Metrnl as a biomarker in RA. Investigating its association with disease progression, inflammation, and cardiovascular risk may provide valuable insights into RA management and risk stratification.

One of the primary limitations of this study is the relatively small sample size. A larger and more diverse cohort could provide a more comprehensive understanding of the associations observed and enhance the generalizability of the findings. A case-control study Design: Longitudinal studies are needed to assess changes over time and determine the impact of interventions on cardiovascular risk in rheumatoid arthritis patients.

Conclusion

Our findings support that Metrnl increased in patients with RA than controls however lower metrnl was correlated with increased carotid intima-media thickness in patients with RA. Age, BMI, disease duration, Family history of premature atherosclerosis, CRP, ESR, TG, cholesterol level, LDL, HDL, and Metrnl were linked to an increase in CIMT in rheumatoid patients. Metrnl was associated with increasing CIMT in rheumatoid patients in multivariate logistic regression analysis; therefore, lower metrnl levels it may be an atherosclerotic risk marker in those patients.

Ethics approval and consent to participate.

The study was approved by the ethical committee of Menoufia University with code 3/2022INTIM42.

Acknowledgements:

This study was carried out by Rheumatology immunology research Committee in Menoufia university and Menouf hospital with appreciated support from Dr. Ahmed El-sabbagh.

Consent for publication: Not applicable

Availability of data and materials

The raw data required to reproduce these findings are available in the body and illustrations of this manuscript.

Author's contribution

The authors confirm contribution to the paper as follows: study conception and design: Sabry S, Emad E.; data analysis and validation: Shimaa S, Sara K, Enas Z,; draft manuscript preparation: Emad E., Shimaa S, Sara K, Enas Z, All authors reviewed the results and approved the final version of the manuscript.

Funding: None

Conflicts of interest:

The authors declare that there is no conflict of interest regarding the publication of this article.

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References:

1. Smolen, J.S., D. Aletaha, A. Barton, G.R. Burmester, P. Emery, G.S. Firestein, A. Kavanaugh, I.B. McInnes, D.H. Solomon, V. Strand, and K. Yamamoto, *Rheumatoid arthritis.* Nat Rev Dis Primers, 2018. **4**: p. 18001.
2. 2 - Kirkeskov, L. and K. Bray, *Employment of patients with rheumatoid arthritis - a systematic review and meta-analysis.* BMC Rheumatology, 2023. **7**(1): p. 41.
3. Rodríguez-Rodríguez, L., R. López-Mejías, M. García-Bermúdez, C. González-Juanatey, M.A. González-Gay, and J. Martín, *Genetic markers of cardiovascular disease in rheumatoid arthritis.* Mediators of inflammation, 2012. **2012**.
4. Santos-Neto, P.J., E.H. Sena-Santos, D.P. Meireles, M.S. Bittencourt, I.S. Santos, I.M. Bensenor, and P.A. Lotufo, *Association of carotid plaques and common carotid intima-media thickness with modifiable cardiovascular risk factors.* Journal of Stroke and Cerebrovascular Diseases, 2021. **30**(5): p. 105671.
5. Hensley, B., C. Huang, C.V. Cruz Martinez, H. Shokoohi, and A. Liteplo, *Ultrasound Measurement of Carotid Intima-Media Thickness and Plaques in Predicting Coronary Artery Disease.* Ultrasound Med Biol, 2020. **46**(7): p. 1608-1613.
6. Gao, X., T.F. Leung, G.W. Wong, W.H. Ko, M. Cai, E.J. He, I.M. Chu, M.S. Tsang, B.C. Chan, J. Ling, X. Fan, L. Lu, C.W. Lam, and C.K. Wong, *Meteorin-β/Meteorin like/IL-41 attenuates airway inflammation in house dust mite-induced allergic asthma.* Cell Mol Immunol, 2022. **19**(2): p. 245-259.
7. Liu N, Dong J, Li L, Zhou D, Liu F. The Function and Mechanism of Anti-Inflammatory Factor Metrnl Prevents the Progression of Inflammatory-Mediated Pathological Bone Osteolytic Diseases. J Inflamm Res. 2024 Mar 11;17:1607-1619. doi: 10.2147/JIR.S455790.
8. Miao, Z.-W., J. Chen, C.-X. Chen, S.-L. Zheng, H.-Y. Zhao, and C.-Y. Miao, *Metrnl as a secreted protein: Discovery and cardiovascular research.* Pharmacology & Therapeutics, 2024. **263**: p. 108730.
9. Gholamrezayi, A., M. Mohamadinarab, P. Rahbarinejad, S. Fallah, S.R. Barez, L. Setayesh, N. Moradi, R. Fadaei, E. Chamani, and T. Tavakoli, Characterization of the serum levels of Meteorin-like in patients with inflammatory bowel disease and its association with inflammatory cytokines. Lipids in health and disease, 2020. **19**: p. 1-8.
10. Aletaha, D., T. Neogi, A.J. Silman, J. Funovits, D.T. Felson, C.O. Bingham III, N.S. Birnbaum, G.R. Burmester, V.P. Bykerk, and M.D. Cohen, *2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative.* Arthritis & rheumatism, 2010. **62**(9): p. 2569-2581.
11. Wang, M., C. Chao, K. Mei, D. Di, Y. Qian, B. Wang, and X. Zhang, *Relationship between rheumatoid arthritis and cardiovascular comorbidity, causation or co-occurrence: A Mendelian randomization study.* Front Cardiovasc Med, 2023. **10**: p. 1099861.
12. Dijkshoorn B, Raadsen R, Nurmohamed MT. Cardiovascular Disease Risk in Rheumatoid Arthritis Anno 2022. J Clin Med. 2022 May 11;11(10):2704. doi: 10.3390/jcm11102704.
13. Gonzalez-Gay, M.A., C. Gonzalez-Juanatey, A. Piñeiro, C. Garcia-Porrua, A. Testa, and J. Llorca, *High-grade C-reactive protein elevation correlates with accelerated atherogenesis in patients with rheumatoid arthritis.* J Rheumatol, 2005. **32**(7): p. 1219-23.
14. Tutoğlu, A., A. Boyaci, N. Boyaci, Z. Kaya, R. Aridici, and I. Koca, *Is There Any Relationship between Joint Destruction and Carotid Intima-media Thickness in Patients with Rheumatoid Arthritis?* J Phys Ther Sci, 2014. **26**(7): p. 1093-6.
15. Cai, J., Q.M. Wang, J.W. Li, F. Xu, Y.L. Bu, M. Wang, X. Lu, and W. Gao, Serum Meteorin-like is associated with weight loss in the elderly patients with chronic heart failure. J Cachexia Sarcopenia Muscle, 2022. 13(1): p. 409-417.
16. Jung, T.W., D.H. Pyun, T.J. Kim, H.J. Lee, E.S. Park, A.M. Abd El-Aty, E.J. Hwang, Y.K. Shin, and J.H. Jeong, Meteorin-like protein (METRNL)/IL-41 improves LPS-induced inflammatory responses via AMPK or PPARδ-mediated signaling pathways. Adv Med Sci, 2021. 66(1): p. 155-161.
17. Dong WS, Hu C, Hu M, Gao YP, Hu YX, Li K, Ye YJ, Zhang X. Metrnl: a promising biomarker and therapeutic target for cardiovascular and metabolic diseases. Cell Commun Signal. 2024 Aug 5;22(1):389. doi: 10.1186/s12964-024-01767-8. PMID: 39103830; PMCID: PMC11301845
18. Naqvi, T.Z. and M.S. Lee, Carotid intima-media thickness and plaque in cardiovascular risk assessment. JACC Cardiovasc Imaging, 2014. 7(10): p. 1025-38.
19. El-Ashmawy, H.M., F.O. Selim, T.A.M. Hosny, and H.N. Almassry, *Association of low serum Meteorin like (Metrnl) concentrations with worsening of glucose tolerance, impaired endothelial function and atherosclerosis.* Diabetes Res Clin Pract, 2019. **150**: p. 57-63.
20. Castaneda, A.B., L.E. Petty, M. Scholz, R. Jansen, S. Weiss, X. Zhang, K. Schramm, F. Beutner, H. Kirsten, U. Schminke, S.J. Hwang, C. Marzi, K. Dhana, A. Seldenrijk, K. Krohn, G. Homuth, P. Wolf, M.J. Peters, M. Dörr, A. Peters, J.B.J. van Meurs, A.G. Uitterlinden, M. Kavousi, D. Levy, C. Herder, G. van Grootheest, M. Waldenberger, C. Meisinger, W. Rathmann, J. Thiery, J. Polak, W. Koenig, J. Seissler, J.C. Bis, N. Franceshini, C. Giambartolomei, A. Hofman, O.H. Franco, B. Penninx, H. Prokisch, H. Völzke, M. Loeffler, C.J. O'Donnell, J.E. Below, A. Dehghan, and P.S. de Vries, *Associations of carotid intima media thickness with gene expression in whole blood and genetically predicted gene expression across 48 tissues.* Hum Mol Genet, 2022. **31**(7): p. 1171-1182.
21. Zhang S, Lei Y, Sun T, Gao Z, Li Z, Shen H. Elevated levels of Metrnl in rheumatoid arthritis: Association with disease activity. Cytokine. 2022 Nov;159:156026. doi: 10.1016/j.cyto.2022.156026. Epub 2022 Sep 7.
22. Garrigues, F., S. Jousse-Joulin, R. Bouttier, M. Nonent, L. Bressollette, and A. Saraux, *Concordance between clinical and ultrasound findings in rheumatoid arthritis.* Joint Bone Spine, 2013. **80**(6): p. 597-603.
23. Oliver, J.E. and A.J. Silman, *Why are women predisposed to autoimmune rheumatic diseases?* Arthritis research & therapy, 2009. **11**(5): p. 1-9.
24. Kłodziński, Ł. and M. Wisłowska, *Comorbidities in rheumatic arthritis.* Reumatologia, 2018. **56**(4): p. 228-233.

25. Ali, R.H., N.A. Fathi, E.M. Abu-Alfadl, and H.S. Mohamed, *Assessment of Joint inflammatory activity in rheumatoid arthritis: a comparative study between clinical evaluation and musculoskeletal ultrasound.* Assessment, 2017. **21**(3).
26. Soliman, A.F., S.E.-S. Egaila, A.I. Ali, N.I. Azab, and H.H. Al-Gohary, *HLA-DRB1 alleles in Egyptian rheumatoid arthritis patients: Relations to anti-cyclic citrullinated peptide antibodies, disease activity and severity.* The Egyptian Rheumatologist, 2016. **38**(4): p. 269-275.
27. Naredo, E., P. Collado, A. Cruz, M.J. Palop, F. Cabero, P. Richi, L. Carmona, and M. Crespo, *Longitudinal power Doppler ultrasonographic assessment of joint inflammatory activity in early rheumatoid arthritis: Predictive value in disease activity and radiologic progression.* Arthritis Care & Research, 2007. **57**(1): p. 116-124.
28. Sakr, B.R., M.M. Elfishawi, M.H. ElArousy, A.K. Hatw, A.N. AbdulKarim, A.B. Tammam, A.N. Kotp, M.E. Hamed, I.E. Genedy, E.D.E. Desouky, and Z.O. Nawito, *Rheumatoid arthritis: A single-center Egyptian experience.* Immunol Invest, 2018. **47**(3): p. 293-302.
29. Arora, A., V. Ingle, R. Joshi, R. Malik, and G. Khandelwal, *Exploring the Subclinical Atherosclerotic Load in Patients With Rheumatoid Arthritis: A Cross-Sectional Study*. Cureus, 2022. 14(12): p. e32644.
30. Zheng, S., Z. Li, J. Song, P. Wang, J. Xu, W. Hu, Y. Shi, Q. Qi, Z. Miao, Y. Guan, and C. Miao, *Endo*thelial METRNL determines circulating METRNL level and maintains endo*thelial function against atherosclerosis.* Acta Pharm Sin B, 2023. 13(4): p. 1568-1587.