RESEARCH ARTICLE

Ibrahim Halil Damar[1]
Recep Eroz[2]

[1]Department of Cardiology, Medical Faculty, Duzce University, Duzce, Turkey
[2]Department of Medical Genetics, Medical Faculty, Aksaray University, Aksaray, Turkey

Corresponding Author:
Ibrahim Halil Damar
mail: ihdamar1@gmail.com

Received: 14.01.2022
Acceptance: 06.03.2022
DOI: 10.18521/ktd.1073568

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

May Argyrophilic Nucleolar Organizer Regions be the New Marker of a Hypoxic Response in Non ST Elevation Myocardial Infarction?

ABSTRACT

Objective: Non-ST elevation myocardial infarction (NSTEMI) is a type of acute coronary syndrome and its' incidence is similarly high to ST-elevation myocardial infarction. Nucleolar organizing regions (NORs) are located of the secondary structure of acrocentric chromosome and composed of proteins and ribosomal DNA (rDNA) some of which are argyrophilic. We aimed to investigate the change of AgNOR proteins, which increase in hypoxia, in patients with NSTEMI.

Methods: A total of 125 participants, 63 patients with NSTEMI and 62 volunteers without any acute coronary syndrome were included in the study. Echocardiography was performed and both mean AgNOR Number and total AgNOR area/total nuclear area (TAA/TNA) were detected for each individuals.

Results: The mean AgNOR number and TAA/TNA ratio were significantly higher in the NSTEMI group than control ($p<0.001$). Also, statistically significant relations between TAA/TNA and all of creatinine, hemoglobin, WBC(µl/ml), monocyte, neutrophil, neutrophil / lymphocyte ratio, monocyte / lymphocyte ratio were detected ($p<0.05$ for all). Also, statistically significant relations between mean AgNOR number and all of fasting blood sugar, HDL, WBC(µl/ml), monocyte, neutrophil, EF were detected ($p<0.001$).

Conclusions: Both AgNOR protein amounts may be used as a marker for NSTEMI. It may also contribute to the prediction of the outcomes by providing some prognostic information in NSTEMI.

Keywords: AgNOR, Hypoxia, NOR, NSTEMI.

Arjirofilik Nükleolar Organize Edici Bölgeler Non ST Elevasyonlu Miyokard İnfarktüsünde Hipoksik Yanıtın Yeni bir Belirteci Olabilir mi?

ÖZET

Amaç: Non-ST elevasyonlu miyokard enfarktüsü (NSTEMI), bir akut koroner sendrom türüdür ve görülme sıklığı ST elevasyonlu miyokard enfarktüsüne benzer şekilde yüksektir. Nükleolar organize edici bölgeler (NORs), akrosentrik kromozomun ikincil yapısında yer alır ve bazıları arjirofilik olan proteinlerden ve ribozomal DNA'dan (rDNA) oluşur. NSTEMI hastalarında, hipokside artan AgNOR proteinlerinin değişimini araştırmayı amaçladık.

Gereç ve Yöntem: Toplam 125 katılımcı, 63 NSTEMI hastası ve herhangi bir akut koroner sendrom tanısı olmayan 62 gönüllü çalışmaya dahil edildi. Bütün hastalara ekokardiyografi yapıldı ve her birey için hem ortalama AgNOR sayısı hem de toplam AgNOR alanı/toplam nükleer alan (TAA/TNA) tespiti yapıldı.

Bulgular: Ortalama AgNOR sayısı ve TAA/TNA oranı NSTEMI grubunda kontrole göre anlamlı derecede yüksekti ($p<0.001$). TAA/TNA ile, kreatinin, hemoglobin, WBC(µl/ml), monosit, nötrofil, nötrofil/lenfosit oranı, monosit/lenfosit oranı arasında istatistiksel olarak anlamlı ilişkiler tespit edildi (tümü için $p<0.05$). Ayrıca ortalama AgNOR sayısı ile, açlık kan şekeri, HDL, WBC(µl/ml), monosit, nötrofil ve EF arasında istatistiksel olarak anlamlı ilişkiler tespit edildi ($p<0.001$).

Sonuç: Her iki AgNOR protein miktarı, NSTEMI için bir markır olarak kullanılabilir. NSTEMI'de bazı prognostik bilgiler sağlayarak sonuçların tahmin edilmesine de katkıda bulunabilir.

Anahtar Kelimeler: AgNOR, Hipoksi, NOR, NSTEMI.

INTRODUCTION

Non-ST elevation myocardial infarction (NSTEMI) is a type of acute coronary syndrome characterized by elevated cardiac biomarkers without persistent ST segment elevation on electrocardiography (1). In line with the increasing incidence of diabetes and the elderly population, the incidence of NSTEMI is similarly high to ST-elevation myocardial infarction (STEMI) (2). Despite many studies to provide primary or secondary prevention, mortality and morbidity rates are not yet at the desired level due to the multitude of gray zones in the pathophysiology of the NSTEMI (3). Many factors that accelerate the development of the disease, especially major risk factors such as diabetes, hyperlipidemia, hypertension, smoking, and genetic predisposition, play an important role in the process starting with endothelial dysfunction and leading to plaque rupture and acute coronary thrombosis (4). It is possible that immune system cells, especially neutrophils, lymphocytes and monocytes, contribute to the pathophysiology with a protective mechanism in the first hours when acute coronary thrombosis begins to occur and inflammation is most active.

Nucleolar organizing regions (NORs) are the locations of ribosomal genes composed of proteins and ribosomal DNA (rDNA) some of which are argyrophilic. After silver staining is applied, NORs can be visualized as localized black spots, particularly with the nucleolar space and are known as "AgNORs". There are studies in the literature emphasizing the importance of AgNOR proteins with various cells such as myocyte (5,6), muscle cells (7), lung cells (8), buccal epithelial cells (9), etc. As a result of these studies, it was determined that the hypoxic state after CO exposure increased the amount of AgNOR protein and that increased AgNOR proteins may have some secondary protective effects against hypoxia (5,6). In NSTEMI, myocardial perfusion is impaired secondary to acute coronary thrombosis, and subsequent ischemia/hypoxia occurs in myocytes. There are no studies in the literature on the evaluation of AgNOR proteins in patients with NSTEMI.

In present study, we aimed to investigate the change in AgNOR proteins in patients with NSTEMI in whom hypoxia is observed significantly. We also aimed to compare AgNOR proteins of NSTEMI subjects to those in control group.

MATERIAL AND METHODS

Study Design: A total of 125 participants, 63 patients who underwent percutaneous coronary intervention with the diagnosis of NSTEMI whom presented to outpatient cardiology clinic of our institution, and 62 volunteers without the diagnosis of any acute coronary syndrome, were included in the study. The study protocol was certified by the local Ethics Committee (ethical approval code:2021/61). Written informed consent was acquired from each participant. Congenital heart disease, severe heart valve disease, atrial fibrillation, hyperthyroidism, hypothyroidism, infection or malignancy were considered as exclusion criteria. The diagnosis of NSTEMI was made according to the 2020 ESC Guidelines for the treatment of acute coronary syndromes in patients without persistent ST-segment elevation (1). Only the responsible lesion was intervened in patients with a diagnosis of NSTEMI who underwent percutaneous coronary intervention. Coronary artery stenosis was determined if the plaques cause 50% or more obstruction in coronary lumen, while hemodynamically insignificant stenosis is determined if the lesions were caused less than 50% stenosis. Blood samples of the patients with a diagnosis of NSTEMI included in the study were obtained within the first 6 hours after the onset of chest pain. Hypertension was defined as the use of antihypertensive drugs or blood pressure ≥140/90 mmHg. Diabetes mellitus was describe as use of antidiabetic therapy or fasting plasma glucose levels > 6.94 mmol/L (>125 mg/dL). Hyperlipidemia was determined as serum total cholesterol ≥5.2 mmol/L, low-density lipoprotein ≥2.6 mmol/L, triglyceride ≥1.7 mmol/L, or using of cholesterol-lowering medication (10). Demographic characteristics and laboratory findings (white blood cells, neutrophil, lymphocyte and monocyte counts, creatinine, total cholesterol, low density lipoprotein (LDL), triglyceride, high density lipoprotein (HDL), hemoglobin levels) of the participants were recorded.

Electrocardiography and Echocardiography: All participants' 12-lead resting ECGs were recorded with the NIHON KOHDEN Cardiofax ECG 1250K model. (filter range, 0.05-150 Hz; AC filter, 60 Hz, 10 mm / mv, 25 mm / sec). Siemens acuson SC 2000 model device was used for echocardiography. Ejection fraction, segmental wall motion abnormality, cardiac anatomy and valve functions were evaluated with standard projections according to the guidelines of the American Society of Echocardiography (11).

Coronary Angiography: Selective right and left coronary angiography and PCI procedures were performed on the patients in the NSTEMI group with the General Electric INNOVA 2100 IQ

model device using the standard Judkin technique. Coronary arteries were visualized at right and left oblique positions with cranial and caudal angles.

AgNOR Staining: Blood samples from both NSTEMI and control groups were distributed on clean slides. After the slides were air-dried for 15 minutes, they were fixed with absolute methanol for 5 minutes at room temperature. Subsequently, silver staining was performed for each slide with the Benn and Perle protocol (12) and slight modification of Lindner (13). The solution prepared by mixing one volume of 1% aqueous formic acid and 2% gelatin with two volumes of 50% silver nitrate was dropped onto the slides and incubated at 37 ^{0}C for 15 minutes in the dark. After incubation process, the slides washed with bi-distilled water were made ready for examination.

Image Analysis of Mean AgNOR Number and Total AgNOR Area/Total Nuclear Area (TAA/TNA) Ratio: First, silver-stained lymphocyte cells from each individual were photographed with a digital camera system (Digital Sight DS-Fi1c; Nikon) attached to a light microscope (Eclipse 80i; Nikon, Tokyo, Japan). Then, using the "free-hand selection" tool for each core, necessary measurements were made using ImageJ version 1.47t image processing software (14) to determine both the TAA/TNA ratio and the average AgNOR number for each core.

Statistical Analysis: The study data were analyzed using the Statistical Package for Social Sciences (IBM Corp., Armonk, NY, USA) 23.0. The data distribution was examined with the Kolmogorov-Smirnov test. Due to the non-normal distribution of the data ($P < 0.05$), non-parametric tests were preferred for statistical analysis. For pairwise comparison of the groups, first descriptive statistics and then Mann-Whitney U tests were used. In addition, polynomial regression test was applied. The $p < 0.05$ was considered statistically significant.

RESULTS

Totally 125 persons (63 with NSTEMI and 62 with control) were included in the current study. The frequency of male and female gender was 68.3%/31.7% and 64.5%/35.5% for the patient and control groups, respectively. There was no significant difference between the two groups in terms of cardiovascular major risk factors except diabetes mellitus. Among the laboratory findings of groups, creatinine (mg/dL), WBC (White Blood Cell) (µl/ml), monocyte, neutrophil, neutrophil/lymphocyte ratio, Monocyte/Lymphocyte ratio were significantly higher in NSTEMI patients than control group ($p<0.05$ for all). On the contrary, HDL (mg/dL) level was significantly higher in the control group ($p=0.001$). The troponin levels of the patients in the NSTEMI group were 5.658 (0.128-21.3) ng/ml. When echocardiographic parameters were compared, ejection fraction was higher in the control group ($p<0.001$), while the interventricular septal thickness and rate of tricuspid regurgitation were significantly higher in the NSTEMI group ($p<0.05$ for all) (Table 1). The ratio of the culprit lesion in NSTEMI patients were 28(44.4%), 17(27%) and 18 (28.6%) for LAD, CX and RCA, respectively.

Also the mean AgNOR number ((1,3 (1-2,9) vs 1 (1-1,4)) and TAA/TNA ratio (0.2 (0,11-0,45) vs 0,05 (0,03-0,1)) were significantly higher in the NSTEMI group than control ($p=0.000$).

When the TAA/TNA ratio to be considered, statistically significant relations between TAA/TNA and all of creatinine, hemoglobin, WBC(µl/ml), monocyte, neutrophil, neutrophil / lymphocyte ratio, monocyte / lymphocyte ratio were detected (Figure1 and Table 2).

Furthermore, when the mean AgNOR number to be considered, statistically significant relations between mean AgNOR number and all of fasting blood sugar, HDL, WBC(µl/ml), monocyte, neutrophil, EF were detected (Figure 2 and Table 3). Silver stained NOR in the lymphocytes of NSTEMI (a, b, c, d), and control (e,f,g,h) groups (X100 magnification) were given in the Figure 3.

DISCUSSION

Striking findings of present study showed that mean AgNOR number and TAA/TNA ratio were significantly higher in the NSTEMI group than in the control group.

In previous studies, it was determined that the hypoxic state due to CO exposure caused an increase in AgNOR proteins (15-18). However, it was reported that the TAA/TNA ratio could be used as an indicator to determine the level of CO exposure resulting in hypoxia (16). It has also been reported that the TAA/TNA ratio can be used as a biomarker instead of histopathological evaluation scores in determining the degree of myocardial damage in rats (5). In addition, it has been shown that some information can be obtained in the detection of cardiomyopathy (CM) levels through AgNOR proteins and that these proteins can be used as an alternative to carboxyhemoglobin (CoHb) to detect CO poisoning levels (6).

Considering the role of lymphocytes in inflammation in the first hours of acute coronary thrombosis and myocardial ischemia/hypoxia in patients with NSTEMI, it is expected that there will be some molecular changes controlling inflammation at the cell nucleus level. In our study, the fact that both the TAA/TNA ratio and mean AgNOR number were higher in patients with NSTEMI compared to the control group can be evaluated in the context of these molecular changes.

Table 1. Clinical, laboratory and echocardiographic findings of both groups

	NSTEMI (n=63) Mean±SD (min-max)	**Control (n=62) Mean±SD (min-max) (median)**	χ^2	**p**
Sex (M/F) (%)	43(68.3%)/20(31.7%)	40(64.5%)/22(35.5%)	0.196	0.658
Diabetes mellitus (Yes/No) (%)	24(38.1%)/39(61.9%)	13(21%)/49(79%)	4.399	0.036
Hypertension (Yes/No) (%)	40(63.5%)/23(36.5%)	35(56.5%)/27(43.5%)	0.645	0.422
Hyperlipidemia (Yes/No) (%)	35(55.6%)/28(44.4%)	36(58.1%)/26(41.9%)	0.080	0.777
FHCD (Yes/No) (%)	20(31.7%)/43(68.3%)	15(24.2%)/47(75.8%)	0.884	0.347
Smoking(Yes/No) (%)	36(57.1%)/27(42.9 %)	18(29%)/44(71%)	10.063	0.002
			Z	**p**
Age (years)	58.03±10.07(41-74) (59)	56.69±11.17(34-75) (57.5)	-0.618	0.537
BMI in Diagnosis (kg/m2)	27.19±1.76(22.46-32.72) (27.04)	27.61±3.56(20.86-37.89) (27.26)	-0.452	0.651
Systolic Blood pressure (mmHg)	134.65±9.981(120-150) (135)	133.95±12.647(100-160) (135)	-0.176	0.860
Diastolic Blood pressure (mmHg)	86.47±6.03(60-100) (90)	86.61±7.933(60-100) (90)	-0.471	0.638
Fasting Blood Sugar	108.32±31.52(70-180) (90)	104.07±22.99(77-200) (99.5)	-0.736	0.462
Creatinin (mg/dL)	0.91±0.20(0.48-1.50) (0.86)	0.83±0.16(0.53-1.27) (0.82)	-2.050	0.040
LDL (mg/dL)	118.08±38.44(35-210) (120)	122.18±34.89(50-201) (123.5)	-0.790	0.429
HDL (mg/dL)	41.90±12.18(23-97) (39)	48.31±12.28(25-82) (45)	-3.254	0.001
Triglyceride(mg/dL)	161.32±88.55(60-509) (144)	153.47±67.08(67-349) (134.5)	-0.040	0.968
Total Cholesterol (mg/dL)	190.60±44.18(100-325) (185)	200.95±41.93(120-305) (202.5)	-1.568	0.117
Hemoglobin (g/dL)	13.64±1.48(10-17) (13.5)	14.08±1.35(11.3-17.2)(13.95)	-1.596	0.111
WBC (µl/ml)	11766.67±3836.16(5400-29300) (11600)	6737.1±1569.19(4300-10600)(6300)	-8.082	<0.001
Platelet ($X10^3$)	269.52±79.23(136-565) (260)	245.13±63.65(149-481) (229.5)	-1.874	0.061
Lymphocyte ($X10^3$)	2.25±1.09(0.32-6.20) (2.3)	2.02±0.52(0.65-3.10) (2.015)	-1.190	0.234
Monocyte ($X10^3$)	0.76±0.35(0.06-1.7) (0.7)	0.51±0.18 (0.21-1.46) (0.5)	-4.567	<0.001
Neutrophil ($X10^3$)	8.54±3.9(2.9-26.50) (7.6)	4.03±1.35(2.09-8.16) (3.66)	-8.061	<0.001
Neutrophil/Lymphocyte	5.56±5.65(1-29.34) (3.36)	2.17±1.1(0.88-7.11) (1.88)	-5.516	<0.001
Monocyte/Lymphocyte	0.44±0.43(0.04-2.80) (0.34)	0.27±0.16(0.13-1.12) (0.026)	-3.163	0.002
Mean AgNOR Number	1.53±0.52(1-2.90) (1.3)	1.08±0.12(1-1.4) (1)	-6.699	<0.001
TAA/TNA	0.21±0.06(0.11-0.45) (0.2)	0.05±0.02(0.03-0.1) (0.05)	-9.645	<0.001
	Echocardiographical Findings			
	STEMI Mean±SD (min-max)	**Control Mean±SD (min-max)**	**Z**	**p**
EF (n %)	50.65±8.18(30-61) (50)	63.87±2.78(50-65) (65)	-9.326	<0.001
IVST (cm)	1.18±0.13(0.9-1.7) (1.2)	1.07±0.14(0.8-1.5) (1)	-4.278	<0.001
	STEMI n(%)	**Control n(%)**	χ^2	**p**
MR (Yes/No) (%)	40(63.5%)/23(36.5%)	30(48.4%)/32(51.6%)	2.893	0.089
AR	12(19%)/51(81%)	10(16.1%)/52(83.9%)	0.184	0.668
PR	10(15.9%)/53(84.1%)	3(4.8%)/59(95.2%)	4.083	0.043
TR	31(49.2%)/32(50.8%)	22(35.5%)/40(64.5%)	2.409	0.121

FHCD: Family History of Cardiovascular Disease, **BMI:** Body mass index, **HDL:** High-density lipoprotein, **LDL:** Low-density lipoprotein, **EF:** Ejection Fraction **MR:** Mitral Regurgitation, **TR:** Tricuspid Regurgitation **AR:** Aortic Regurgitation **PR:** Pulmonary Regurgitation, **LAD** :Left Anterior Descending Artery, **LCX:** Left Circumflex Artery **RCA**: Right Coronary Artery, **Min-Max:** Minimum-Maximum**, SD:** Standard deviation, ***=**Statistically significant, **IVST**: interventricular septum thickness, **WBC:**White Blood Cell

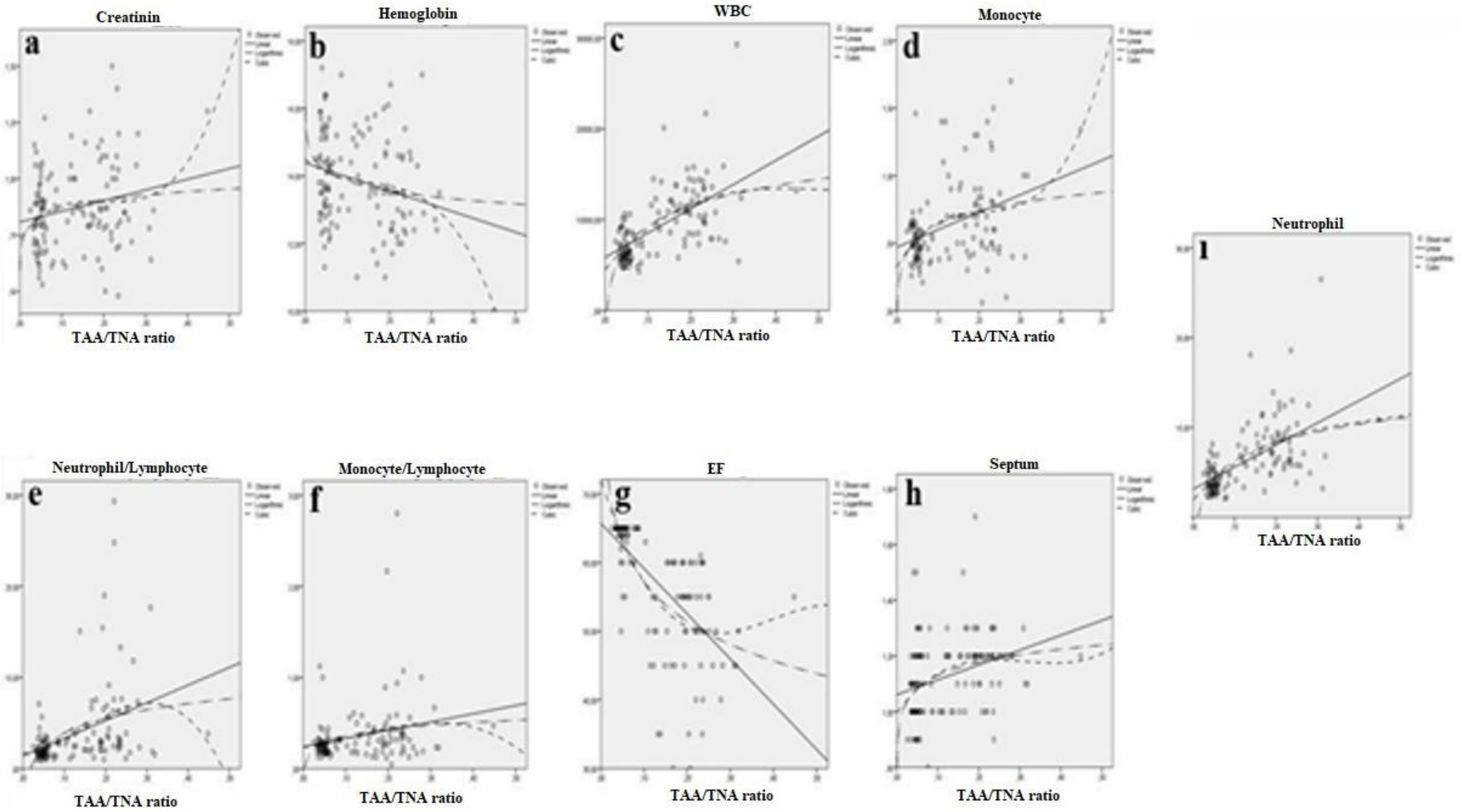

Figure 1. The relation between creatinine and TAA/TNA ratio (a), hemoglobin and TAA/TNA ratio (b), WBC and TAA/TNA ratio (c), monocyte and TAA/TNA ratio (d), Neutrophil / Lymphocyte ratio and TAA/TNA ratio (e), monocyte / Lymphocyte ratio and TAA/TNA ratio (f), EF and TAA/TNA ratio (g), Septum and TAA/TNA ratio (h), neutrophil and TAA/TNA ratio (ı) for both groups.

Table 2. Model Summary and Parameter Estimates for TAA/TNA and all of creatinin, hemoglobin, WBC, monocyte, neutrophil, Neutrophil / Lymphocyte, Monocyte / Lymphocyte, EF, IVST of both groups

		Model Summery					**Parameter Estimates**			
Variable	***Equation***	***R^2***	***F***	***df1***	***df2***	***sig***	**Constant**	***b1***	***b2***	***b3***
Creatinin and TAA/TNA	Linear	0.049	6.297	1	123	0.013	0.808	0.475		
	Log	0.043	5.577	1	123	0.020	0.991	0.053		
	Cubic	0.067	2.887	3	121	0.038	0.733	2.612	-13.487	22.710
Hemoglobin (g/dL) and TAA/TNA	Linear	0.064	8.428	1	123	0.004	14.385	-4.098		
	Log	0.049	6.306	1	123	0.013	12.882	-0.422		
	Cubic	0.091	4.031	3	121	0.009	14.499	-10.038	54.043	-118.653
WBC(µl/ml) and TAA/TNA	Linear	0.370	72.208	1	123	<0.001	5833.129	26694.334		
	Log	0.382	76.079	1	123	<0.001	16668.897	3204.454		
	Cubic	0.392	26.037	3	121	<0.001	4478.610	54671.794	-108456.9	68986.829
Monocyte and TAA/TNA	Linear	0.137	19.546	1	123	<0.001	0.470	1.287		
	Log	0.130	18.397	1	123	<0.001	0.977	0.148		
	Cubic	0.155	7.405	3	121	<0.001	0.320	5.387	-24.579	39.227
Neutrophil and TAA/TNA	Linear	0.343	64.348	1	123	<0.001	3.139	24.554		
	Log	0.349	65.813	1	123	<0.001	13.045	2.921		
	Cubic	0.359	22.552	3	121	<0.001	1.978	49.205	-101.313	80.073
Neutrophil / Lymphocyte and TAA/TNA	Linear	0.151	21.895	1	123	<0.001	1.374	19.458		
	Log	0.156	22.729	1	123	<0.001	9.270	2.335		
	Cubic	0.177	8.671	3	121	<0.001	1.690	2.191	160.047	-354.778
Monocyte / Lymphocyte and TAA/TNA	Linear	0.056	7.279	1	123	0.008	0.240	0.905		
	Log	0.056	7.357	1	123	0.008	0.604	0.107		
	Cubic	0.062	2.685	3	121	0.050	0.235	0.679	3.761	-10.236
EF and TAA/TNA	Linear	0.414	86.725	1	123	<0.001	65.687	-65.816		
	Log	0.470	109.203	1	123	<0.001	38.073	-8.290		
	Cubic	0.484	37.772	3	121	<0.001	71.984	-200.729	565.007	-473.520
IVST and TAA/TNA	Linear	0.107	14.686	1	123	<0.001	1.061	0.533		
	Log	0.124	17.351	1	123	<0.001	1.286	0.068		
	Cubic	0.127	5.891	3	121	0.001	0.995	2.026	-6.893	7.386

IVST: Interventricular septum thickness, **EF:** Ejection Fraction, **WBC:**White Blood Cell

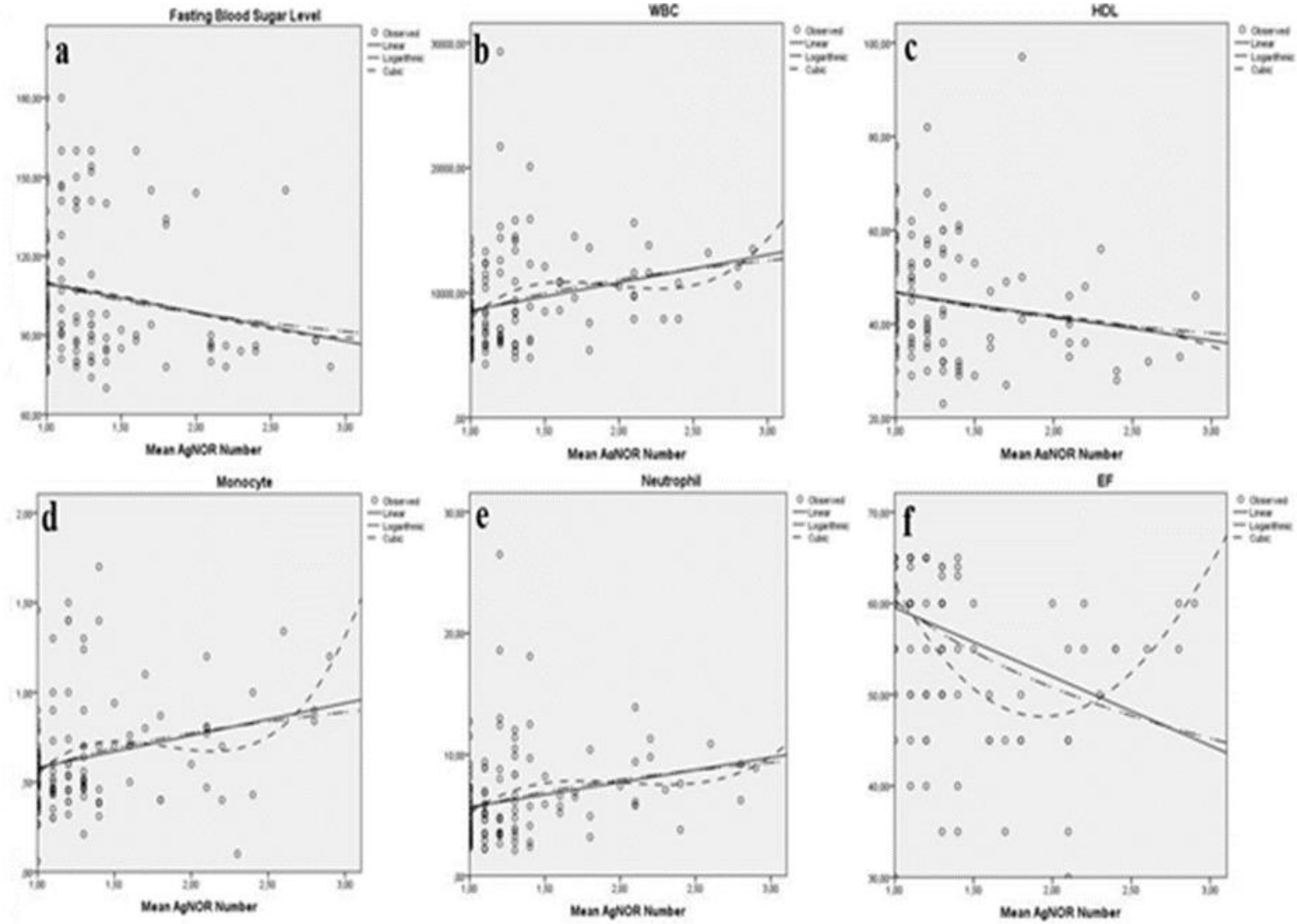

Figure 2. The relation between fasting blood sugar and mean AgNOR number (a), WBC and mean AgNOR number (b), HDL and mean AgNOR number (c), monocyte and mean AgNOR number (d), neutrophil and mean AgNOR number (e) EF and mean AgNOR number (f) for both groups.

Table 3. Model Summary and Parameter Estimates for Mean AgNOR number and all of fasting blood sugar, HDL, WBC, monocyte, neutrophil, EF of both groups

		Model Summery					**Parameter Estimates**			
Variable	***Equation***	***R^2***	***F***	***df1***	***df2***	***sig***	**Constant**	***b1***	***b2***	***b3***
Fasting Blood Sugar and Mean AgNOR	Linear	0.030	3.853	1	123	0.052	120.502	-10.928		
	Log	0.029	3.688	1	123	0.057	110.025	-16.950		
	Cubic	0.031	1.275	3	121	0.286	102.664	21.572	-18.220	3.175
HDL and Mean AgNOR	Linear	0.032	4.005	1	123	0.048	51.730	-5.084		
	Log	0.031	3.995	1	123	0.048	46.892	-8.045		
	Cubic	0.032	1.328	3	121	0.268	61.187	-22.707	10.162	-1.824
WBC(µl/ml) and Mean AgNOR	Linear	0.064	8.445	1	123	0.004	6359.450	2226.720		
	Log	0.074	9.825	1	123	0.002	8419.444	3785.483		
	Cubic	0.098	4.374	3	121	0.006	-20093.4	49688.181	-26078.23	4445.557
Monocyte and Mean AgNOR	Linear	0.067	8.771	1	123	0.004	0.401	0.179		
	Log	0.070	9.209	1	123	0.003	0.570	0.291		
	Cubic	0.104	4.674	3	121	0.004	-1.962	4.642	-2.615	0.477
Neutrophil and Mean AgNOR	Linear	0.059	7.718	1	123	0.006	3.637	2.038		
	Log	0.069	9.057	1	123	0.003	5.518	3.479		
	Cubic	0.085	3.763	3	121	0.013	-16.638	37.671	-19.036	3.141
EF and Mean AgNOR	Linear	0.136	19.387	1	123	<0.001	67.095	-7.559		
	Log	0.179	26.866	1	123	<0.001	60.303	-13.742		
	Cubic	0.298	17.147	3	121	<0.001	113.230	-71.435	21.264	-0.968

IVST: Interventricular septum thickness, **EF:** Ejection Fraction , **WBC:**White Blood Cell

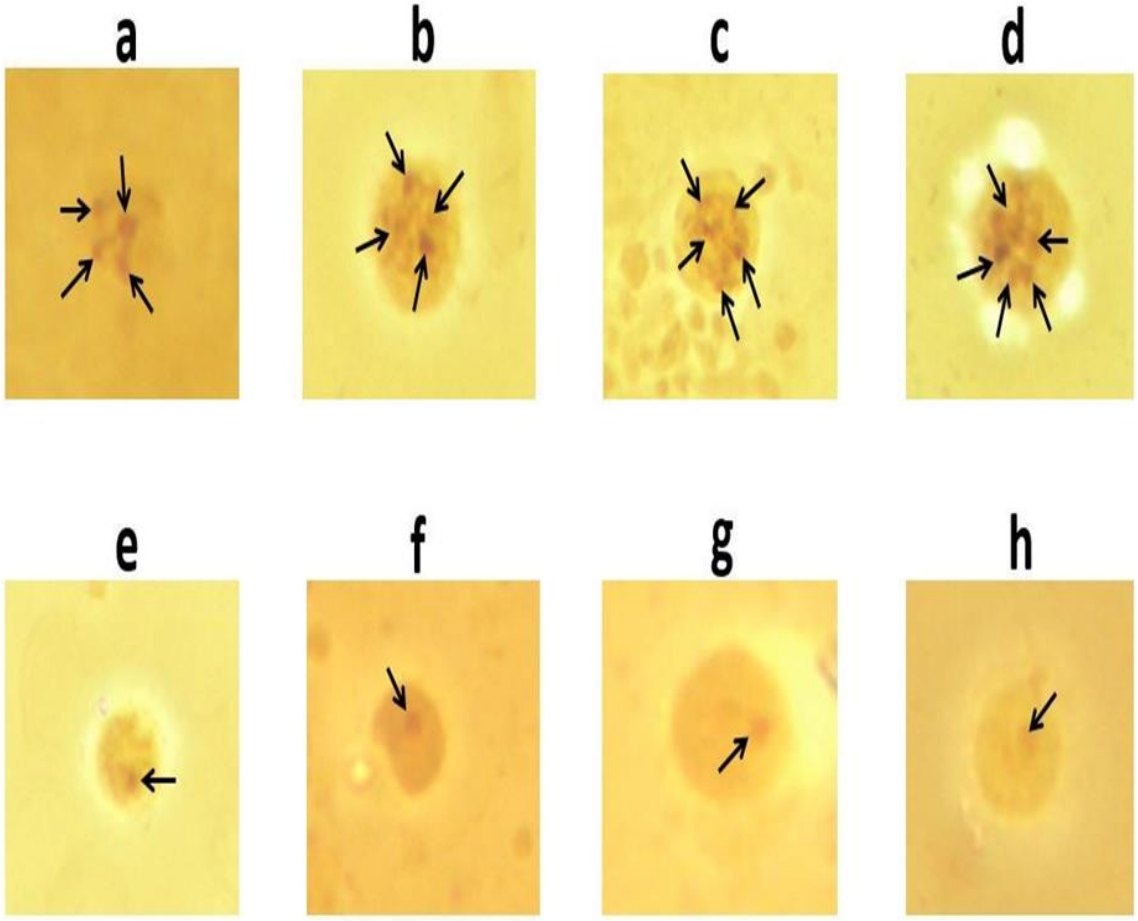

Figure 3. Silver stained NOR in the lymphocytes of NSTEMI (a, b, c, d), and control (e,f,g,h) groups (X100 magnification)

In addition, we found that statistically significant relationship between TAA/TNA and all of creatinine, hemoglobin, WBC (µl/ml), monocytes, neutrophils, neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio and statistically significant relationship between AgNOR count and all of fasting blood glucose, HDL, WBC (µl/ml), monocytes, neutrophils, and EF indicate that the effects of AgNOR proteins cannot be ignored in the acute coronary syndrome process. In other words, the significant relationship between AgNOR proteins and cardiovascular risk factors such as high blood sugar, low HDL, anemia, and creatinine and the significant relationship between AgNOR proteins and inflammatory cells such as monocytes and neutrophils, which have very important functions in acute coronary syndrome (3,17,18) seem remarkable.

Until now, many studies have been conducted in the literature regarding the prognostic value of parameters such as WBC count, monocyte count, neutrophil count, neutrophil/lymphocyte ratio, Monocyte/Lymphocyte ratio in patients with acute coronary syndrome (18-22). In our study, we observed that these parameters were parallel to the literature data in patients with NSTEMI and we also witnessed a statistically significant relationship with AgNOR proteins.

Relatively small study cohort and single center nature of the study design are limitaions of present work. Yet, this is the first study in literature reported increased AgNOR in NSTEMI subjects.

Conclusion

Both AgNOR protein amounts may be used as a marker for NSTEMI. It may also contribute to the prediction of the outcomes by providing some prognostic information in NSTEMI.

Conflict of Interest: The authors declare that there is no conflict of interest

Funding: There is no funding

Acknowledgements: We thank the participants for their contribution.

Author Contributions: IHD and RE: Coordination of the study, Manuscript writing, database management, echocardiography data analysis, IHD and RE: Data collection, statistical analysis, IHD and RE: Contribution to the concept, design and critical revision of article.

REFERENCES

1. Collet JP, Thiele H, Barbato E, Barthélémy O, Bauersachs J, Bhatt DL, et al. ESC Scientific Document Group. 2020 ESC Guidelines for the management of acute coronary syndromes in patients

presenting without persistent ST-segment elevation. Eur Heart J. 2021:42(14):1289-1367. Erratum in: Eur Heart J. 2021;42(19):1908. Erratum in: Eur Heart J. 2021;42(19):1925. Erratum in: Eur Heart J 2021.
2. Awan MS, Daud MY, Khan M, Jehangiri AU, Adnan, Jalil S. Usefulness Of Neutrophils To Lymphocytes Ratio For Predicting Troponin-I Elevation In Patients Presenting With Suspected Nste-Acute Coronary Syndrome. J Ayub Med Coll Abbottabad 2019; (Suppl 1)(4):S674-S677.
3. Chen H, Li M, Liu L, Dang X, Zhu D, Tian G. Monocyte/lymphocyte ratio is related to the severity of coronary artery disease and clinical outcome in patients with non-ST-elevation myocardial infarction. Medicine (Baltimore) 2019; 98(26).
4. Damar İH, Eroz R. The Role of Macrophages in Atherosclerosis: An Overview. Erciyes Med J. 2021;43(1): 9–12.
5. Saritas A, Gunes H, Colakoglu S, Eroz R, Akoz A, Oktay M, et al. Are there any effects of chronic carbon monoxide exposure on argyrophilic nucleolar-organizing region-associated protein synthesis in rat myocardium? Hum Exp Toxicol. 2016;35(9):921-8.
6. Kandiş H, Afacan MA, Eröz R, Colakoglu S, Bayramoglu A, Oktay M, et al. Can argyrophilic nucleolar organizing region-associated protein amount be used for the detection of cardiac damage? Hum Exp Toxicol. 2016;35(3):323-31.
7. Gunes H, Saritas A, Eroz R, Colakoglu S. Use of argyrophilic nucleolar–organizer regionassociated protein synthesis in skeletal muscle cells for prediction of chronic carbon monoxide exposure. Toxin Reviews. 2020;39(4):349-354.
8. Çolakoğlu S, Saritas A, Eroz R, Oktay M, Yaykasli KO, Akoz A, et al. Is one-time carbon monoxide intoxication harmless? Evaluation by argyrophilic nucleolar-organizing regions staining method. Hum Exp Toxicol. 2015;34(1):24-31.
9. Selvi B, Demirtas H, Eroz R, Imamoglu N. Reduction of the argyrophilic nucleolar organizing region associated protein synthesis with age in buccal epithelial cells of healthy individuals. Aging Clin Exp Res. 2015;27(2):201-8.
10. Damar İH, Eroz R. The Association of Hereditary Prothrombotic Risk Factors with ST-Elevation Myocardial Infarction. Medeni Med J. 2020;35(4):295-303.
11. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2015; 28(1):1-39.
12. Benn PA, Perle M. Chromosome staining and banding techniques. In: Rooney, DE, Czepulkowski, BH (eds). Human Cytogenetics, Constitutional Analysis, practical approach, Vol 1. Oxford: Oxford University Press. 1986, pp.91–118.
13. Lindner LE. Improvements in the silver-staining technique for nucleolar organizer regions (AgNOR). J Histochem Cytochem. 1993 41(3):439-45.
14. Rasband WS. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997 –2016.
15. Nisari M, Eroz R, Nisari M, Ertekin T, Oktay M, Kavutcu M. Investigation of argyrophilic nucleolar organizing region. Bratisl Lek Listy. 2016;117(6):345-50.
16. Eroz R, Saritas A, Colakoglu S, Oktay M, Kandiş H. Evaluation of argyrophilic nucleolar organizing region–associated protein synthesis in femoral muscle cells of rats exposed 3000 ppm carbon monoxide gas. Konuralp Medical Journal 2016; 8: 9-13.
17. Caimi G, Lo Presti R, Canino B, Ferrera E, Hopps E. Behaviour of the neutrophil to lymphocyte ratio in young subjects with acute myocardial infarction. Clin Hemorheol Microcirc. 2016;62(3):239-47.
18. Park JS, Seo KW, Choi BJ, Choi SY, Yoon MH, Hwang GS, et al. Importance of prognostic value of neutrophil to lymphocyte ratio in patients with ST-elevation myocardial infarction. Medicine (Baltimore). 2018;97(48).
19. Fan Z, Li Y, Ji H, Jian X. Prognostic utility of the combination of monocyte-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio in patients with NSTEMI after primary percutaneous coronary intervention: a retrospective cohort study. BMJ Open. 2018;8(10).
20. Misumida, Naokia; Kobayashi, Akihiroa; Kanei, Yumikob Racial differences in the neutrophil-to-lymphocyte ratio in patients with non-ST-segment elevation myocardial infarction, Coronary Artery Disease: 2015 - Volume 26 - Issue 5 - p 381-385
21. Guo TM, Cheng B, Ke L, Guan SM, Qi BL, Li WZ, et al. Prognostic Value of Neutrophil to Lymphocyte Ratio for In-hospital Mortality in Elderly Patients with Acute Myocardial Infarction. Curr Med Sci. 2018;38(2):354-359.
22. Meeuwsen JAL, Wesseling M, Hoefer IE, de Jager SCA. Prognostic Value of Circulating Inflammatory Cells in Patients with Stable and Acute Coronary Artery Disease. Front Cardiovasc Med. 2017; 14;4:44.