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Study of rs1137101 polymorphism of leptin receptor gene with serum levels of selenium and copper in the patients of non-ST-segment elevation myocardial infarction (NSTEMI) in an Iranian population

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ABSTRACT

Objective: NSTEMI is a type of myocardial infarction (MI) causing partial but progressive occlusion of cardiac coronary vessels. The aim of this study was to investigate rs1137101 polymorphism of soluble leptin receptor (sLEPR) as well as circulatory selenium and copper levels in NSTEMI patients and their usefulness in analyzing susceptibility to NSTEMI.

Methods: We collected sera and whole blood of 80 NSTEMI patients and 80 healthy individuals using cTnI levels plus electrocardiography as the "gold standard". Polymorphism analysis was done after DNA extraction by high-resolution melt PCR, selenium and copper levels by atomic absorption spectrophotometry, and sLEPR by ELISA.

Results and discution: There was Hardy-Weinberg (HWE) equilibrium for both patient and control loci ($\chi 2 = 0.368434509$ and 0.341447368, respectively). The frequencies of A/A, A/G, and G/G genotypes were 18 (22%), 37 (46%), and 25 (31%) for patients, and 30 (38%), 36 (45%), and 14 (18%) for healthy controls, respectively. The frequencies of A and G alleles were 73 (46%) and 87 (54%) for patients and 96 (60%) and 64 (40%) for control groups. There was correlation between allele G and sLEPR level and Body Mass Index (BMI). Selenium levels were lower in patient group than control group (66.307 ± 11.013 against 87.488 ± 11.839 μg/L; $p < 0.001$) but copper concentrations were higher (1.8105 ± 0.358 against 1.366 ± 0.454 mg/L; $p < 0.001$). sLEPR levels were also higher in patient than control group (30.568 ± 3.290 against 23.740 ± 5.457 ng/dL; $p < .001$). Low selenium and high copper concentration had positive diagnostic value for disease.

Conclusion: We find for the first time that there is a significant association between rs1137101 polymorphism and susceptibility to NSTEMI. There is also statistically meaningful association between decrease in serum selenium and increase in serum copper levels with susceptibility to NSTEMI.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide [1], with rapidly rising incidence in low- and middle-income countries [2], including Iran [3]. Coronary artery disease (CAD) is most common and serious type of CVDs and myocardial infarction (MI) is in turn the most severe type of CADs. Along with history taking and physical examination, troponin I and T, and less often creatine kinase MB (CK-MB) [4] are used to diagnosis of MI, and electrocardiography (ECG) to observe ST-segment change or no-change is applied to distinguish between its main subtypes ST elevation MI (STEMI) and non-ST elevation MI (NSTEMI), respectively [5]. The infracted coronary artery is occluded partially in NSTEMI, although it is almost totally occluded in STEMI. The importance of timely diagnosis and proper recognition and management of NSTEMI is that partial coronary obstruction in NSTEMI could be a basis for accumulation of excessive materials, which results in complete obstruction of it and result in serious heart attack. Proper and fast diagnosis would lead to

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appropriate treatment and reduce mortality rate [6]. Leptin (LEP or OB) an adipocyte-specific hormone that regulates adipose tissue mass through its effect on the hypothalamus acts through leptin receptor (LEPR or OBR) a membrane of cytokine receptors [7]. Soluble LEPR (sLEPR) could also bind leptin and modulates its function [8]. Increase in sLEPR has been demonstrated in CVDs [9].

rs1137101 (Gln223Arg) polymorphism is a single nucleotide polymorphism (SNP) in nucleotide A to G which causes replacement of glutamine by arginine at amino acid 223 in the final sequence of protein.

Selenium is a necessary micronutrient in mammals, included in more than twenty-five selenoproteins like glutathione peroxidases, iodothyronine deiodinases, selenophosphate synthetase 2, and thioredoxin reductases [10]. The normal concentration of selenium in the serum is 70–150 μg/L (ng/mL). High and low serum concentrations of selenium leads to selenosis and selenium deficiency, that the later have been reported in various disorders including: Kashin-Beck [11], AIDS [12], diabetes mellitus [13] and CADs in general [14] but not specific for NSTEMI that is studied in the current approach.

Copper, another essential micronutrient for the health, is included in metaloenzymes like amine oxidases, ceruloplasmin, cytochrome c oxidase, dopamine β-hydroxylase, hephaestin, lysyl oxidase, superoxide dismutase (Cu, Zn), and tyrosinase [15]. Increase or decrease from its standard serum concentration (0.637–1.4012 mg/L or 63.7–140.12 μg/dL) causes its toxicity or deficiency, respectively. Along with hereditary deficiencies of copper (Menke's and Wilson diseases), copper deficiency had been seen in disorders like: osteoporosis and osteoarthritis, [16], colon and prostate cancer [17], chronic diseases of bone and connective tissue [18], and cardiovascular diseases in whole [19] but not specific for NSTEMI that is investigated in this research. The objectives of this study were evaluating rs1137101 polymorphism as well as sLEPR, selenium, and copper difference between NSTEMI and control groups, calculating the effects of rs1137101 polymorphism on LEPR, and investigating the diagnostic roles of selenium and copper levels for susceptibility to NSTEMI.

As an appealing new field of science, the Molecular Pathologic Epidemiology (MPE) tries to join molecular pathology (e.g SNP) to epidemiologic factors in order to precise measure of interfering factors of the disease [20, 21]. There is remarkable works of Shuji Ogino and coworkers in the field of integrative studies between endogenous and exogenous factor of cancers (especially colon cancer) and immune diseases for precise differentiation of intercalating factors of the diseases [22–24]. We analyzed the interaction of rs1137101 polymorphism (as endogenous factor) with selenium and copper (as exogenous factors) as an MPE approach.

2. Materials and methods

2.1. Ethics statement

Everyone participated in this study signed a written consent. The obtained information were kept confidential. We applied WMA Declaration of Helsinki as well as the guidelines of the Medical Ethics Committee of Tabriz University of Medical Sciences (Ethic Code: IR.TBZMED.REC.1395.43; April 24, 2016) in this study. Blood sampling was completed done with relevant rules of the hospital.

2.2. Study population

80 patients (40 males and 40 females) attended to the emergency room with diagnosis of NSTEMI were selected and blood sampling was fulfilled. 80 healthy people (40 males and 40 females) with normal cTnI levels (the gold standard) as the control group. Patients with history of stroke, malignancy, muscle injury, trauma, cirrhosis, renal diseases, infectious diseases, serum albumin levels < 2 g/dL, hypertension, and diabetes mellitus were excluded from study. Patients complaining of chest pain and dyspnea were referred to serum cTnI measurements. Serum cTnI levels ≤ 0.05 ng/L were diagnosed as Unstable Angina (UA) and did not enter this study. Then electrocardiography was performed on patients with cTnI levels above 0.05 ng/L (MI patients), and subjects who did not have elevated ST segment were enrolled in current study as NSTEMI patients.

2.3. Sample collection and storage

Mediplus clot-activator containing tubes (Sunphoria Co. Ltd., Taiwan) were used to collect blood samples. After 10 min of incubation in room temperature for coagulation, serum was separated by centrifugation for 7 min at 10000 rpm and transferred to RNase/DNase-free tubes and stored frozen at −80 °C (snijders scientific, The Netherlands) until analysis.

2.4. DNA isolation and preparation

DNA was extracted using GeneMatrix Quick Blood DNA Purification Kit (cat. No. E3565; EurX). The kit uses a Spin-Column and extraction buffers for taking out DNA from serum after degradation of RNAs by RNase A and proteins by proteinase K. The quality of extraction was assessed by Nanodrop model ND-1000 spectrophotometry (Wilmington, USA). Extracted DNA samples were kept frozen at −80 °C (snijders scientific, The Netherlands).

2.5. Polymorphism study by qPCR

Primers were designed by Primer3 Online Software [25], considering the study of Yang et al. In 2016 [26] as follows:

Forward 5′-TCCTGCTTTAAAAGCCTAATCCAGTATTT-3′.

Reverse 5′-AGCTAGCAAATATTTTTGTAAGCAAT-3′.

and their quality was analyzed by Primer-BLAST Online Software [27]. 5× HOT FIREPol® EvaGreen® HRM Mix (no ROX) (Solis BioDyne, Estonia) was used as master mix. Thermocycler conditions were set as follows: 1) Initial denaturation at 95 °C for 15 min, 2) 40 cycles at 95 °C for 15 s, 60 °C for 20 s and 72 °C for 20 s, and 3) Melt with a gradient of 0.3 °C/s. The PCR product is a DNA fragment of 367 nucleotides that contains the genome template from nucleotide 65,592,588 to nucleotide 65,592,928 in chromosome 1. The location of polymorphism rs1137101 is at 65592830 template genome. The confirmation of PCR product genotypes was performed by electrophoresis on agarose gel. The MspI restriction enzyme was selected for this purpose via the NEBcutter v 2.0 online program [28]. By choosing a random series of 8 patient and 8 control samples and sequencing, the A and G loci were located.

2.6. Detection of soluble leptin receptor

To measure sLEPR concentrations in serum samples of the studied groups, an ELISA kit from Zellbio, Germany was used and kit instructions were followed.

2.7. Measurement of cTnI

Chemiluminescent analyzer model Architect i1000 SR (Abbott, USA) was applied and commercial kits from Pars Azmoon (Tehran, Iran) was used to measure cTnI concentration.

2.8. Measurement of serum selenium and copper levels

Selenium was extracted by Clinton et al. method [29]. In this method, nitric acid (HNO3), hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2) were used. The method presented by Ichida et al. In 1969 [30], which is dilution of serum samples in 1:1 proportion by with iron-free water, was used to prepare copper samples before quantitative

analysis. CTA 3000 Atomic Absorption Spectrophotometer (ChemTech Analytical Instruments Limited, UK) was used to determine both selenium and copper concentrations in sera samples. The extraction processes caused selenium and copper dilution 35.7 and two times, respectively. Therefore the numbers obtained for selenium and copper from the atomic absorption apparatus were multiplied to 35.7 and 2, respectively.

2.9. Measurement of other analytes of the study

We quantified all other analytes of the current study the auto-analyzer Autolab, BT 3500 Auto analyzer Medical System (Rome, Italy) using commercially used kits in clinical laboratory of Seyedoshohada Cardiovascular Medical Hospital.

2.10. Statistical analysis

We used Microsoft Excel version 2016 (Microsoft Corp., Redmond, WA, USA) and XLSTAT Extension Version 2016.02.28451 (Addinsoft, Paris, France) to statistical analysis. A confirmatory Kolmogorov–Smirnov normality test (K-S test) was done by SPSS version 20 (SPSS Inc., Chicago, IL, USA) software to validity normal distribution of studied groups. All statistical tests were analyzed two-tailed and p values below 0.05 were considered significant. Pearson's correlation coefficient was assigned as the measurant for correlation studies. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) amounts were determined by Receiver Operating Characteristic Curve (ROC Curve) analysis. Odds ratio (OR) and 95% confidence interval (CI) that represent the risk of NSTEMI and ANOVA tests were done also by SPSS.

3. Results

3.1. Subject characteristics

Table 1 shows a brief review of clinical findings for the studied groups.

We purposely chose studied groups as they had not statistically significant differences in age, sex, and BMI. Although significant differences were seen in CKMB, urea, creatinine, glucose, K+, cholesterol, triglyceride, and ALT levels, the rest of studied chemistries had not.

Table 1
Clinical characteristics of the studied groups.

Characteristics	NSTEMI Patients (n = 80)	Healthy controls (n = 80)	p value (Two-tailed *t*-Test)
Age (years)	62.40 ± 9.86	59.61 ± 10.83	0.091
Male/female	40/40	40/40	1
Body mass index (kg/m2)	25.12 ± 4.57	24.89 ± 4.24	0.617
cTnI (ng/L)	18.90 ± 48.68	0.13 ± 0.11	0.001
CKMB (IU/L)	32.87 ± 22.53	17.92 ± 6.21	< 0.001
Urea (mg/dL)	37.47 ± 14.70	28.56 ± 7.12	< 0.001
Creatinine (mg/dL)	1.15 ± 0.29	1.06 ± 0.15	0.009
Glucose (mg/dL)	144.26 ± 65.96	103.85 ± 17.50	< 0.001
Na^+ (mEq/l)	140.97 ± 1.60	140.88 ± 1.58	0.729
K^+ (mEq/l)	4.17 ± 0.34	4.24 ± 0.32	0.194
Cholesterol (mg/dL)	182.92 ± 37.44	165.35 ± 22.59	< 0.001
TG (mg/dL)	183.70 ± 50.43	160.22 ± 36.02	0.001
HDL (mg/dL)	39.17 ± 8.39	39.51 ± 9.36	0.811
AST	24.81 ± 27.39	25.70 ± 21.37	0.820
ALT	22.86 ± 14.68	30.86 ± 25.94	0.018
ALP	214.36 ± 51.52	200.83 ± 51.68	0.099
LDL	94.26 ± 40.13	98.53 ± 38.12	0.491
TSH	3.69 ± 11.64	2.60 ± 9.09	0.511
FT4	1.80 ± 1.78	2.02 ± 1.90	0.443

cTnI = cardiac troponin I, CKMB = Creatine Kinase-MB isozyme, Na^+ = sodium, K^+ = potassium, TG = Triglyceride, HDL = high density lipoprotein, AST = aspartate transaminase, ALT = alanine transaminase, ALP = alkaline phosphatase, LDL = low-density lipoprotein, TSH = Thyroid-stimulating hormone, FT4 = free thyroxine.

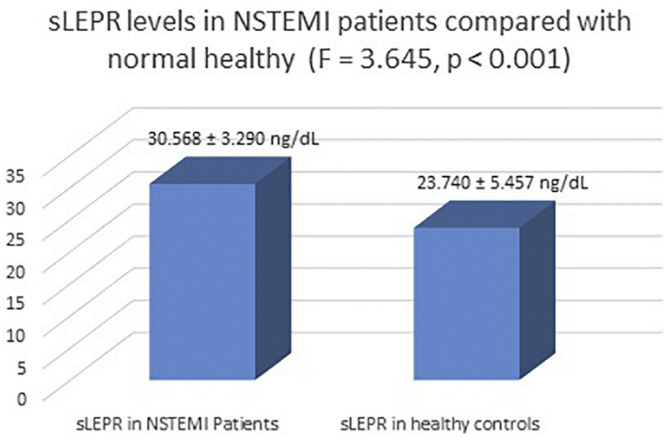

Fig. 1. sLEPR concentrations in the studied groups (p < 0.001).

3.2. Serum level of sLEPR

sLEPR concentration was higher in NSTEMI patients (30.568 ± 3.290 ng/dl) than normal healthy individuals (23.740 ± 5.457 ng/dl) (F = 3.645, p < 0.001). Fig. 1.

3.3. rs1137101 Polymorphism

To evaluate the Hardy-Weinberg (HW) equilibrium for allele and genome proportions, we used a Chi-square goodness-of-fit test. The results of the test showed that there was HW equilibrium for both patient and control loci ($\chi 2 = 0.368434509$ and 0.341447368, respectively). Genotype and allele frequencies were presented in Table 2, and their association with disease and sLEPR level was shown in Tables 3 and 4, respectively.

The results of these tables show that there is higher expression of genotype G/G and allele G in STEMI patients. Furthermore, genotype G/G is associated with NSTEMI in codominant, dominant, and recessive models and genotype A/G in dominant model. Moreover, the relationship between rs1137101 polymorphism and sLEPR concentration is significant in codominant (p = 0.041) and recessive (p = 0.011) models. The differences were more than double when there is a G allele (2.08 (CI = 0.16–3.99) and 2.11 (CI = 0.50–3.73)), respectively.

3.4. Serum level of selenium and copper

There was a diminish in selenium (66.307 ± 11.013 vs. 87.488 ± 11.839 μg/L; p < 0.001) but increase in copper levels (1.8105 ± 0.358 vs. 1.366 ± 0.454 mg/L; p < 0.001) of NSTEMI

Table 2
Genotype and allele frequencies in the studied groups.

Population	N	Genotype	No. observed (%)	Allele frequency	
				A	G
Patient	80	A/A	18 (22%)	73 (46%)	87 (54%)
		A/G	37 (46%)		
		G/G	25 (31%)		
Control	80	A/A	30 (38%)	96 (60%)	64 (40%)
		A/G	36 (45%)		
		G/G	14 (18%)		

Table 3
rsl137101 polymorphism association with response Status of individuals.

Model	Genotype	Control (%)	Patient (%)	OR (95% Cl)	P-value	AIC	BIC
Codominant	A/A	80 (37.5%)	18 (22.5%)	1	0.045	221.6	230.8
	A/G	36 (45%)	37 (46.2%)	1.71 (0.81–3.60)			
	G/G	14 (17.5%)	25 (31.2%)	2.98 (1.24–7.16)			
Dominant	A/A	30 (37.5%)	18 (22.5%)	1	0.038	221.5	227.6
	A/G – G/G	50 (62.5%)	62 (77.5%)	2.07 (1.03–4.13)			
Recessive	A/A- A/G	66 (82.5%)	55 (68.3%)	1	0.042	221.7	227.3
	G/G	14 (17.5%)	25 (31.2%)	2.14 (1.02–4.52)			
Overdominant	A/A- G/G	44 (55%)	43 (53.3%)	1	0.87	225.3	231.9
	A/G	36 (45%)	37 (46.27a)	1.05 (0.56–1.96)			
Log-additive	–	–	–	1.72 (1.11–2.67)	0.013	219.6	225.8

Table 4
rsll37101 association with response sLEPR (adjusted by Status).

Model	Genotype	n	Response mean (s.e)	Difference (95% Cl)	P-value	AIC	BIC
Codominant	A/A	48	25.86 (0.82)	0	0.041	937.2	952.6
	A/G	73	26.66 (0.6)	−0.06 (−1.69–1.57)			
	G/G	39	29.68 (0,93)	2.08 (0.16–3.99)			
Dominant	A/A	48	25,86 (0.82)	0	0.41	941,1	953.4
	A/G – G/G	112	27.71 (0,52)	0.65 (−0.89–2.20)			
Recessive	A/A-A/G	121	26.34 (0.49)	0	0.011	935.2	947.5
	G/G	39	29.68 (0.93)	2.11 (0.50–3.73)			
Overdominant	A/A- G/G	87	27.57 (0.65)	0	0.17	939.8	952.1
	A/G	73	26.66 (0.6)	−0.99 (−2.39–0.40)			
Log-additive	–	–	–	0.99 (0.03–1.95)	0.045	937.7	950

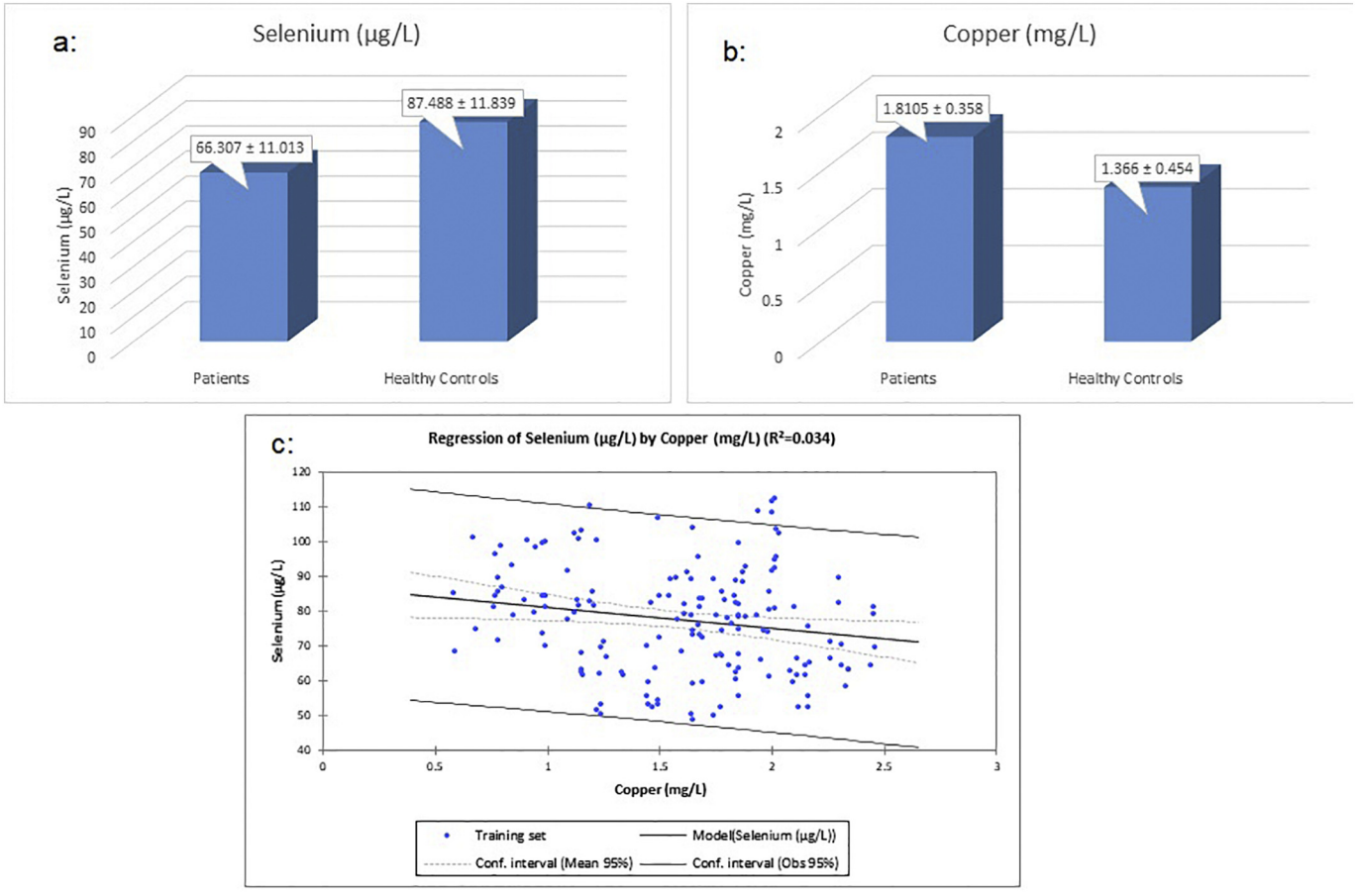

Fig. 2. A: selenium and B: copper levels in the studied groups, and C: their correlation.

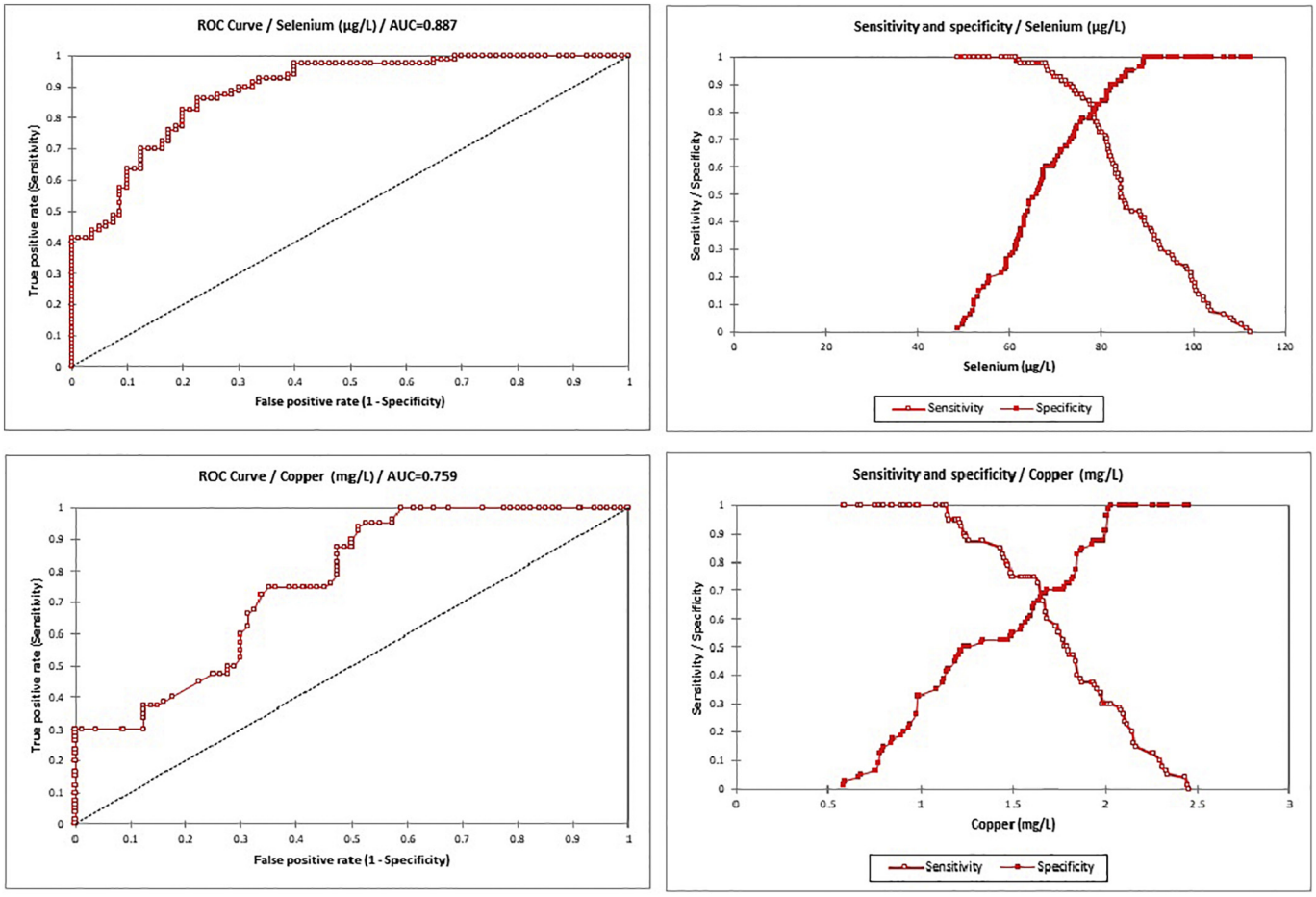

Fig. 3. The diagnostic value of selenium and copper for NSTEMI disease.

Table 5
Descriptive analysis of correlation of rs1137101 polymorphism with selenium/copper.

	Genotype	N of genotype	Mean selenium	Mean copper
Patients	AA	18	68.7794 ± 10.77	1.8239 ± 0.39
	AG	36	64.1986 ± 10.53	1.8494 ± 0.36
	GG	26	70.7065 ± 11.19	1.7046 ± 0.35
Controls	AA	31	85.5184 ± 10.42	1.3677 ± 0.47
	AG	36	86.5125 ± 12.58	1.3439 ± 0.44
	GG	13	93.5369 ± 13.14	1.4823 ± 0.45

patients comparing to healthy controls. There was a positive correlation between diminish in selenium and increase in copper levels in NSTEMI disease p value = 0.020; $R^2 = 0.034$; the correlation formula is Selenium (µg/L) = 87.0029–6.0479 × Copper (mg/L). Fig. 2.

Table 7
MPE relations by ANOVA analysis between copper and rs1137101 polymorphism.

ANOVA							
Copper and rs1137101 polymorphism							
			Sum of squares	df	Mean square	F	Sig.
Between groups	(Combined)		0.233	2	0.116	0.536	0.586
	Linear term	Unweighted	0.215	1	0.215	0.989	0.321
		Weighted	0.222	1	0.222	1.025	0.313
		Deviation	0.010	1	0.010	0.047	0.829
Within Groups			33.853	156	0.217		
Total			34.086	158			

Table 6
MPE relations by ANOVA analysis between selenium and rs1137101 polymorphism.

ANOVA							
Selenium and rs1137101 polymorphism							
			Sum of squares	df	Mean square	F	Sig.
Between groups	(Combined)		573.465	2	286.732	1.230	0.295
	Linear term	Unweighted	37.421	1	37.421	0.161	0.689
		Weighted	59.580	1	59.580	0.256	0.614
		Deviation	513.885	1	513.885	2.205	0.140
Within groups			36,359.490	156	233.074		
Total			36,932.955	158			

3.5. The diagnostic value of selenium and copper for NSTEMI disease

Sensitivity, specificity, and area under the ROC curve (AUCs) of selenium and copper as the diagnostic tests for NSTEMI disease were determined by the ROC curve analysis. The results of the ROC curve analysis showed that the results for selenium showed sensitivity of 86.3%, specificity of 77.5%, and AUC of 0.887 (CI 95% was 0.340–0.434; $p < 0.001$). The results of sensitivity, specificity, and AUC for copper were 95.0%, 48.8% and 0.759, respectively (CI 95% was 0.188 to 0.331; $p < 0.001$). Fig. 3.

3.6. Interaction of rs1137101 polymorphism with selenium and copper

ANOVA using Tukey HSD test by SPSS software didn't show correlation between genotypes (A/A, A/G, and G/G) with serum level of selenium (combined, unweighted, and weighted p values were 0.295, 0.689, and 0.614, respectively) and with copper (combined, unweighted, and weighted p values were 0.586, 0.321, and 0.313, respectively) (Tables 5, 6, 7).

4. Discussion

Study on rs1137101 polymorphism and serum selenium and copper in NSTEMI patients was a part of a generalized study of us about biomarkers of NSTEMI disease, which also included microRNA-4478 [31] and ischemia modified albumin plus protein carbonyl [32]. Coronary artery diseases (CADs) are the most common and serious class of cardiovascular diseases (CVDs). Among CADs, myocardial infarction (MI) has the biggest rate of morbidity and mortality. NSTEMI is a type of MI that causes partially occlusion of infarcted artery, despite STEMI that causes almost complete occlusion.

Timely and proper diagnosis and Management of NSTEMI is important because partial obstruction in NSTEMI can be settled as a basis for accumulation of excessive material, which results in complete obstruction of the engaged vessel and severe heart attack. Different biomarkers like cardiac troponins (I and T) and muscle creatine kinase (CKMB) are now used as recognition markers for MI and its subtypes. sLEPR binds to leptin in ratio of 1:1 and modulates its biochemical action [8]. Previous studies showed increase in sLEPR levels in CVD patients [9], confirmed in the current study.

Polymorphism study is a favorite subject in CADs, and 45 PubMed-indexed studies in various polymorphisms was fulfilled just in 2016 [33]. In rs1137101 (Q223R) polymorphism, an A-to-G shift in nucleotide 668 of the cDNA results in the replacement of glutamine with arginine at its 223 protein chain [34]. This polymorphism plays a role in body mass index, fat mass, and serum leptin levels [35], insulin response to oral glucose tolerance test [36], bone mineral content, and regional bone mineral density [37]. Given the importance of Q223R polymorphism in obesity and heart disease, we decided to test possible Q223R polymorphism as an indicator for predicting NSTEMI. Therefore, the objectives of this study were: 1. Evaluation of difference in Q223R polymorphism in NSTEMI patients and healthy controls, 2. Determine whether this differs from the Hardy-Weinberg equilibrium or not, 4. Calculation of the effects of Q223R polymorphism changes on sLEPR level, and 5. Investigating the role of Q223R polymorphism as one of the predictors of NSTEMI. In the present study, we showed that: 1. There is a significant increase in serum LEPR levels in patients with NSTEMI ($p < 0.001$), 2. The association between rs1137101 polymorphism and sLEPR concentration is significant ($p = 0.041$) in codominant and recessive models. Also, the difference is more than double when there is a G allele (CI 0.16–3.99). 3. Relationship between rs1137101 polymorphism and BMI, and 4. The presence of G allele (and eventually arginine amino acid) in the rs1137101 polymorphism increases the risk of NSTEMI disease. Odds Ratio, which directly indicates the ratio of the disease, is in the presence of G allele (arginine amino acid) in the corresponding polymorphism region, with nearly 95% confidence interval between 1.24 and 7.16, indicating that the presence of G alleles increased The risk of NSTEMI disease (P value = 0.045). Changes in serum selenium levels in reported in cardiovascular diseases [14, 38, 39], however has not been evaluated specifically in NSTEMI. Similarly, changes in serum copper levels in reported in cardiovascular diseases [39–41] but not separately in NSTEMI. The results of this study showed that: 1. There is a significant reduction in selenium levels ($p < 0.001$) but 2. Increase in copper in NSTEMI patients ($p < 0.001$), 3. The association between reduction of selenium and increase in copper in NSTEMI patients is significant ($p = 0.020$), 4. Both selenium and copper has high sensitivity specificity for NSTEMI disease, 5. There was a negative correlation between serum selenium (μg/L) and IMA (ABSU) ($R2 = 0.099$; $p = 0.001$).

5. Conclusion

Statistical interpretations showed for the first time that there is a significant increase in G allele (and hence, arginine) in NSTEMI cases, which is possibly associated with increase in sLEPR concentration. Since sLEPR and mentioned biological agents are important for pathophysiology of NSTEMI as a cardiac disease, so with confirming the increase in the concentration of sLEPR, the Q223R polymorphism could be presented as a new biomarker for NSTEMI disease. We also concluded that Q223R polymorphism as well as decrease in selenium and increase in copper levels could be used a prognostic biomarkers for NSTEMI. Because of some limitations including case and control sample sizes and expensiveness of reagents and kits, and although there was normal distribution in patient and control groups, but we have to confess that small number of subjects was a limitation of our paper and higher sample sizes could gain better conclusions.

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