

CD14 gene polymorphism 159C/T in a group of patients with coronary artery disease from a population with high morbidity of cardiovascular diseases

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Abstract

Background: A role of CD14 receptor in the inflammatory response is stimulation of monocytes and endothelial cells by lipopolysaccharide of Gram-negative bacteria. The reports about association of progression of atherosclerosis with CD14 gene polymorphism in different populations are conflicting.

Aim: To assess if T to C exchange at position 159 of the CD14 gene correlates with age at the onset of first myocardial infarction (MI), severity of coronary atherosclerosis and number of risk factors in MI survivors in a local community characterised by high morbidity of cardiovascular diseases and whether this genotyping could be helpful in identifying patients with a high risk of MI at young age and beyond low number of risk factors.

Methods: Fifty-seven MI survivors (75.5% males) from 98 consecutive patients (pts) with coronary artery disease were included. The genotypes in position 159 of the CD14 gene were determined by polymerase chain reaction. The medical history concerning diabetes mellitus, arterial hypertension, dyslipidaemia, smoking and obesity was taken from every participant. Gensini score (GS) was calculated on the basis of coronarography. Age at first MI, value of GS and number of risk factors were analysed variables. The pts were divided into the decades of life, according to cumulated number of risk factors and into the terciles according to GS. Distribution of ages at first MI, pts with different number of risk factors and percent of pts belonging to determined terciles of GS were compared between subgroups with genotype CC and CT, TT.

Results: The CC genotype was detected in 25 (43.8%) pts, CT in 30 (52.6%) and TT in 2 (3.6%). Age at first MI ranged from 40 to 75 years, mean 58.7 ± 7.23 , values of GS ranged from 0 to 154, mean 48.6 ± 25.7 , and number of risk factors from 0 to 4, mean 1.92 ± 0.99 . No significant differences in distribution of ages at first MI, values of GS or number of risk factors were found between patients with CC and with CT or TT genotype in position 159 of CD14 receptor genotype.

Conclusion: These data indicate that screening for CD14 159C/T polymorphism is unlikely to be a useful tool for risk assessment of MI at young age, independently of low number of risk factors, in a population with high morbidity from cardiovascular diseases.

Key words: CD14 polymorphism, myocardial infarction, screening tests

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Introduction

The population of Łódzkie province, similarly to inhabitants of Świętokrzyskie and Lubelskie provinces, has the highest cardiovascular mortality rate in Poland per 100,000 population, with the percentage of deaths

from ischaemic heart disease (IHD) in 2002 in this region being 25.4% in males and 16.3% in females [1]. The causes of this situation are probably complex: external sociocultural and socioeconomic factors should be considered (quality and availability of medical care), as

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well as independent factors such as genetic determinants of IHD. For many years there has been an increasing number of known alleles for which there is a constant search for their linkage with early development of atherosclerosis, and alleles suspected of association with increased susceptibility to plaque deterioration. Different variants of those alleles, if sufficiently frequent in the population and without signs of spontaneous mutations, are referred to as gene polymorphism, which has been described for a number of genes associated with development of arterial hypertension, presence of dyslipidaemia, obesity and hypercoagulability – risk factors of atherosclerosis known for a long time.

Polymorphism is also observed in genes related to development of inflammatory reactions, including the inflammatory response to Gram-negative bacteria lipopolysaccharide (LPS) associated with atherogenesis – mainly *Chlamydia pneumoniae* and *Helicobacter pylori*. The role of chronic infections in epidemiology of cardiovascular disease is questionable in populations of highly developed countries; however, in populations or ethnic groups of lower socioeconomic status this issue becomes more vital. It is connected to a higher incidence of atherosclerotic diseases and coexistence of chronic inflammatory conditions of the upper respiratory tract, poor condition of dentition and chronic inflammation of oral tissues and more often gastric or duodenal ulcer disease in those groups.

Our studies showed that concomitant infection with *Ch. pneumoniae* and *H. pylori* was associated with strong humoral reaction against antigens of these bacteria and correlated with coronary artery disease (CAD) [2]. One of the stages initiating an inflammatory reaction is the action of LPS (a component of Gram-negative bacteria wall) on CD14 receptors located in cell membranes of monocytes and endothelium or on soluble receptors (sCD14). These receptors act together with Toll-like 4 receptor (TLR4) in launching an inflammatory cytokine cascade, activation of monocytes and interaction between leukocytes and endothelium, as well as in regulation of programmed cell death (apoptosis). These reactions are accelerated by lipopolysaccharide binding protein (LBP). There are also reports available on the role of C-reactive protein or heat shock protein (hsp-60) in activation of this receptor. [3] Serum concentrations of sCD14 as well as the extent of expression of cellular type of CD14 receptor and individual susceptibility of host cells to respond to LPS may result from CD14 receptor gene polymorphism.

Two forms of CD14 receptor (a glycoprotein consisting of 356 amino acids) gene polymorphism have been

identified so far. The first one involves a C-to-T transition at bp-260 (260 C/T) – many publications from 1999 – 2004 described this abnormality [4-8]. Hubacek et al. showed higher incidence of T allele in position 260 in Czech patients who survived myocardial infarction (MI) and also more pronounced expression of CD14 membrane receptor (mCD14) on monocytes in patients with TT genotype compared to CT or CC genotypes [9]. Ito et al. did not confirm a relationship between polymorphism at 260 position and cerebral artery atherosclerosis in a Japanese population [10].

Due to the discrepancy of results between different ethnic groups interest focused on another polymorphism of the CD14 receptor gene involving a C-to-T transition at bp-159 (159C/T). Eilertsen et al., who investigated normal homozygotes with respect to the T allele, found they had lower serum levels of total cholesterol, LDL cholesterol and apolipoprotein B-100 [11]. On this basis it seems that 159C/T polymorphism may be an important genetic factor that determines binding of CD14 to lipids as well as affects their transport.

A paper by Unkelbach et al. regarding the relationship between 159C/T polymorphism of the CD14 receptor gene and CAD in residents of Germany documented that TT homozygotes were more often individuals over 62 years of age with low risk of MI assessed on the basis of accumulation of typical risk factors of atherosclerosis (mainly normotensive non-smoking patients) [12]. The results of this study left investigators a fascinating issue to be solved: does TT genotype have protective action against atherosclerosis since it reveals with this genetic variant only in subjects aged more than the mean age of the study group, or is it more a proatherogenic factor – does this pathology of coronary arteries manifest with this variant despite a small number of typical risk factors?

For better understanding of genetic mechanisms underlying individual susceptibility to the action of proinflammatory factors which may increase the risk of CAD, the current study focused on 159C/T polymorphism in cardiac patients selected from a population with unfavourable cardiovascular epidemiological indices.

The study aimed to evaluate if 159C/T polymorphism of the CD14 receptor gene promotor region in a population with high morbidity and mortality due to CAD was associated with the clinical profile of patients who survived their first MI, and particularly if one of three genotype variants correlated with the age at the time of first infarct, number of identified risk factors or progression of atherosclerosis, scored on the basis of coronary angiography.

Methods

Patients

Of 89 consecutive patients with IHD diagnosed and treated at the Department of Cardiology of Medical University in Łódź in 2003, 57 post-MI individuals were selected. Males comprised 75.5% of studied patients. Age of patients after MI was between 40 and 75 years, mean 59.7 ± 7.4 years. Study group characteristics including presence of typical risk factors of CAD are shown in Table I. The presence of risk factors was established according to the following criteria: arterial hypertension – taking hypotensives prescribed prior to enrolment into the study or systolic blood pressure >140 or diastolic blood pressure >90 mmHg; diabetes – taking oral glucose lowering agents or insulin prior to enrolment, double checks of fasting glucose ≥ 126 mg/dl (7 mmol/l), glycosylated haemoglobin $>7\%$ or positive oral glucose tolerance test with 75 g glucose; dyslipidaemia: taking hypolipaeic agents before enrolment, total cholesterol level 190 mg/dl (5.0 mmol/l) or more, LDL cholesterol >115 mg/dl (3.0 mmol/l), HDL cholesterol <40 mg/dl (1.0 mmol/dl) in males and <46 mg/dl (1.2 mmol/l) in females, or triglycerides >150 mg/dl (1.2 mmol/l); obesity – BMI (body mass index) >32 , nicotineism – confirmed smoking more than 5 cigarettes per day. Coronary angiography reports of each patient were used to calculate Gensini score according to

Table I. Traditional risk factors of atherosclerosis in the study group patients

Risk factor	Percentage of patients
Arterial hypertension	57.9
Diabetes mellitus	26.3
Dyslipidaemia	57.9
Nicotineism	36.8
Obesity	10.5

the description given by Gensini et al. [13]. The index was calculated as a sum of multipliers dependent on degree of lumen narrowing (A) and localisation of atherosclerotic plaque (B) – Table II.

Genetical assessment

Nucleotide test at bp-159 of the CD14 receptor gene was performed using polymerase chain reaction as described by Karhukorpi et al. using two different reaction starters relevant for detection of C and T alleles [14]. DNA was derived from blood leukocytes collected into tubes with EDTA as an anticoagulant. Products of PCR were separated with agarose gel (2%) electrophoresis with addition of ethidium bromide. The strip of 381 bp was identified as corresponding with T allele and 227 bp as corresponding with C allele.

Table II. Calculation of Gensini score as a sum of A and B product for all plaques found on coronary angiography depending on severity of stenosis of the respective segment of coronary artery (A) and plaque location (B)

A		×	B	
Degree of coronary artery stenosis and its score equivalent			Location of plaque and its score equivalent	
0%	0		II Dg from LAD	0.5
25%	1		Postero-lateral branch	
50%	2		Proximal RCA	
			Mid RCA	
			Distal RCA	
			I Dg from LAD	1.0
75%	4		Distal LAD	
			Distal LCx	
			Marginal branch from LCx	
			Posterior descending artery	
90%	8		Mid LAD	1.5
99%	16		Proximal LAD	
		Proximal LCx	2.5	
		Left main coronary artery	5.0	
100%	32			

Abbreviations: Dg – diagonal branch, RCA – right coronary artery, LAD – left anterior descending branch, LCx – left circumflex branch

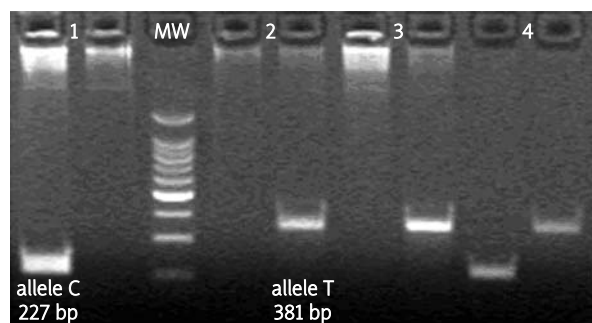


Figure 1. PCR products for detection of 159C/T polymorphism of CD14 receptor gene, separated in 2% agarose gel with addition of ethidium bromide

381 bp product – T allele

227 bp product – C allele

DNA samples: 1 – homozygotic patient – CC (presence of C allele only); 2,3 – homozygotic patient – TT (presence of T allele only); 4 – heterozygotic patient – CT (presence of C and T allele); MW – reference mass pattern

Examples of T and C allele identification and CC, CT and TT genotypes are shown in Figure 1.

Statistical analysis

Mean age at MI, distribution of patients with respect to decades of life at which MI occurred and percentage of patients in who developed MI before the age of 50 or after 70 years were compared to establish whether the tested genotype was associated with age at time of first MI. The relationship of cumulated traditional risk factors of atherosclerosis with studied genotype was verified by calculating mean number of classical risk factors of atherosclerosis for one subject in subgroups after division into three genotypes and numerical distribution of patients free of risk factors, and with one, two, three or more risk factors as well as percentage of patients with fewer than 2 risk factors in compared subgroups. Evaluation of 159T/C CD14 polymorphism association with severity of atherosclerosis was expressed quantitatively as the Gensini score. Patients were divided into tertiles with respect to the above variable and incidence rate of subjects with studied genotypes was compared within individual tertiles.

Results are presented as mean $\pm$ SD or numbers and percentages. Student's t-test was used to contrast mean variables, χ^2 test to compare variables distribution, and u test to evaluate two incidence rates of one feature. A p value <0.05 was considered significant.

The study protocol was approved by the Local Bioethics Committee – resolution on medical experiment project no. RNN/187/2001/KE. All patients gave their written informed consent to participate in the study.

Results

Of 89 patients with IHD, CC genotype was found in 34.8%, CT genotype in 58.4%, and TT genotype in 6.8% of subjects, whereas in patients who survived MI it was found in 25 (43.8%), 30 (52.6%) and 2 (3.6%) subjects, respectively.

Age of patients at the time of their first MI episode ranged from 40 to 75 years (mean 58.7 ± 7.23 years). Number of cumulated risk factors in the study group was from 0 to 4 (mean 1.92 ± 0.99), and Gensini score was between 0 and 154 (mean 48.6 ± 25.7).

Due to the very small number of patients with TT genotype (2 individuals) they were combined with the CT subgroup in analyses. Analysis of age at first MI compared between CC subgroup and both CT and TT subgroup (CT/TT) showed no statistically significant differences. Mean age at first MI episode was 58.7 ± 6.4 in the CC genotype group and 60.6 ± 8.1 in the CT/TT group (NS). Also, neither numerical distribution following matching to decade of age at which MI occurred nor percentage of patients who suffered from MI before their 50s or not until their 70s differed with respect to the studied genotypes (Figure 2).

The compared subgroups did not differ significantly either when analysed regarding the presence of cumulated risk factors of atherosclerosis. Both comparison of percentage distribution of patients with no, one, two, three or four risk factors, as well as comparison of mean number of cumulated risk factors/person (1.88 ± 1.07 for CC genotype vs. 1.95 ± 0.93 for CT/TT genotype) and comparison of percentage of patients with fewer than 2 risk factors per person (36% vs. 31%, respectively) showed no significant differences (Figure 3).

Gensini score as a measure of severity of atherosclerosis of coronary arteries ranged in the entire group from 0 to 154, in the CC subgroup from 11 to 140, and in the CT/TT subgroup from 0 to 154. Tertiles of Gensini score had the following borderline values: I tertile: 0-34; II tertile: 35-50; III tertile: 51-154. Mean Gensini score in the CC genotype group was 49.2 ± 26.8 , and in CT/TT subgroup 48.1 ± 24.8 (NS).

Neither the percentage distribution of patients with CC and CT/TT genotypes in individual tertiles nor the percentage of subjects who due to the result of the Gensini score were assigned to the last (III) tertile differed significantly between analysed groups (Figure 4).

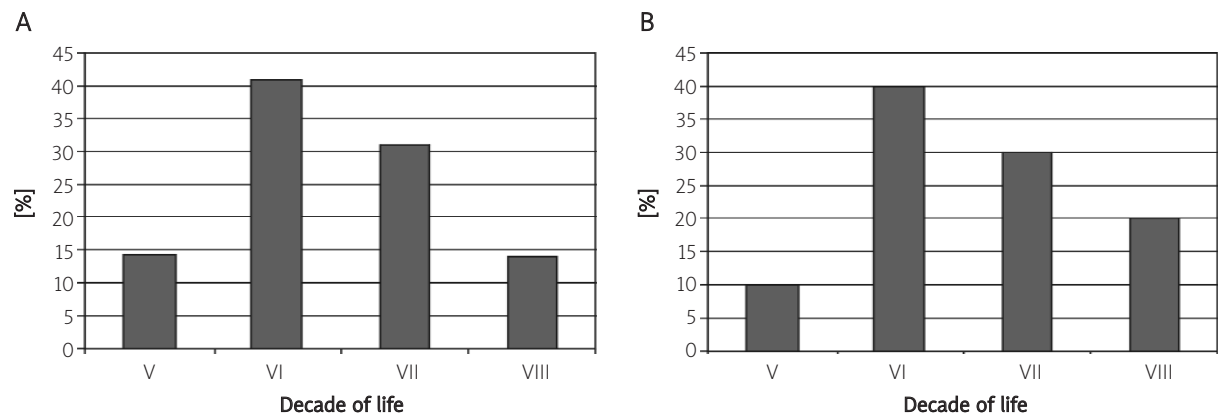


Figure 2. Comparison of patients percentage distribution with respect to age at first MI episode (decade of life) with division to genotype of promotor region of CD14 receptor gene – CC genotype (A), CT and TT genotypes (B) (all differences NS)

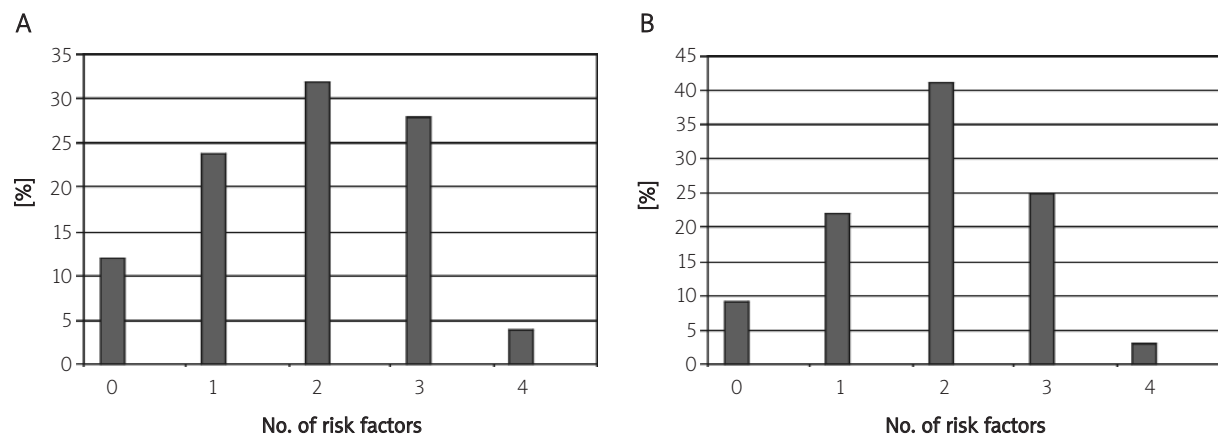


Figure 3. Comparison of percentage distribution of patients with past myocardial infarction with increasing number of cumulated risk factors with respect to type of promotor region genotype of CD14 receptor gene – CC genotype (A), CT and TT genotypes (B) (all differences NS)

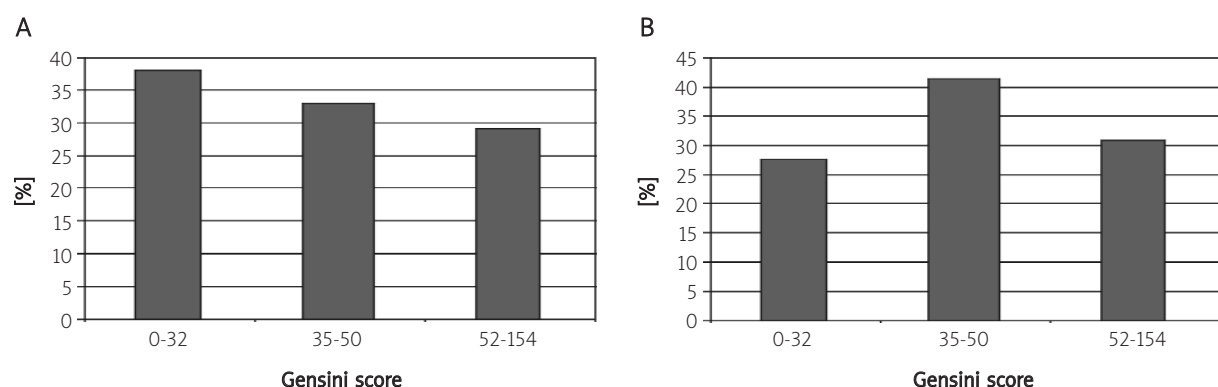


Figure 4. Comparison of percentage distribution of patients with past myocardial infarction with respect to tertile of Gensini score as measure of severity of atherosclerosis of coronary arteries, depending on promotor region genotype of CD14 receptor gene – CC genotype (A) vs. CT and TT genotypes (B) (all differences NS)

Discussion

The problem of 159C/T polymorphism of the CD14 receptor gene and its association with the presence of coronary atherosclerosis or instability was investigated in a few previous studies in various ethnic groups. However, no data for the Polish population have been published so far. In the previously mentioned paper of Unkelbach et al., a genetic probe was used to examine nearly 2300 German patients referred for coronary angiography. Only TT homozygotes were characterised by a specific profile of atherosclerotic risk: individuals with MI occurring at older age and small number of risk factors. The remaining genotypes (CC or CT) were not found to have a relationship with age at MI episode [12]. In the mentioned paper the percentage of patients with TT genotype was 22%, and with CC genotype 28%, whereas the CT genotype was the most common and present in 50% of study participants.

A similar profile of genotype distribution was observed by Koch et al., who investigated different patients from Germany [15]. In our study group with a history of MI, TT genotype occurred markedly less frequently, only in 4% of patients. The distribution of 159C/T polymorphism was similar in non-cardiologic groups from the same population of residents of Łódzkie province. For instance, the frequency of TT genotype did not exceed 5% in patients with dyspepsia (68-subject group), and in 35 normal volunteers TT genotype occurred in 9% of subjects [16, 17]. Thus, in groups selected from a population characterised by unfavourable indices of cardiovascular morbidity and mortality there is a trend towards less frequent incidence of TT genotype, which in the case of a casual relation with atherosclerosis would speak for its protective rather than proatherogenic role.

Opposite conclusions were reached by Chinese investigators who compared 162 patients with CAD with nearly 200 healthy subjects [18]. According to Li et al. the presence of a single T allele increases the risk of CAD by 1.2 times and in homozygotes (TT) this risk was 2.3-fold compared to healthy individuals. In those studies the incidence rate of TT genotype in patients selected from a population living around Wuhan was 38.7%, and in a group with CAD it was 27.0%, so in both cases this variant occurred markedly more often than in our patients [18].

Available published data from other studies indicate some possible pathologic mechanisms determining severity of inflammatory processes depending on genetic variant of CD14 receptor. Losito et al. suggest that 159C/T polymorphism may determine the ability of CD14 to transport cholesterol [19]. The authors assumed that subjects with CC genotype had poor action of mecha-

nisms involved in removal of bacterial endotoxins, and in this way cytokines released from host cells may present increased atherogenicity. Rupp et al. documented that the presence of T allele (CT or TT genotype) increased by 1.7 times susceptibility to infection of monocytes with *Ch. pneumoniae*, a Gram-negative bacterium producing LPS, for which most evidence was gathered implying its relationship with early development and instability of atherosclerotic plaques [20]. On the other hand, Karhukorpi et al. showed the influence of chronic *H. pylori* infection (also producing LPS in the cardiovascular system) on plasma soluble CD14 receptor (sCD14); however, the highest concentrations were observed in individuals with CT and TT genotypes [14].

Our own studies showed that IHD patients infected with *H. pylori* had higher levels of LBP and sCD14 than healthy subjects from the control group [17, 21]. Additionally, it was observed that in IHD patients, but not in normal individuals, with CT/TT genotype, sCD14 levels were higher than in CC patients. This suggests rather indirect regulation of LBP and sCD14 production in patients with CAD and *H. pylori* infection. We may suppose that intense production of both proteins reflects body response to *H. pylori* components, including but not limited to LPS. Our earlier serological studies confirmed very high incidence of *H. pylori* infections in patients with IHD as well as their particular predisposition to potent humoral reaction to *H. pylori* antigens [2, 22]. Elevated levels of sCD14 were observed also during some other bacterial infections, including tuberculosis, independently of genotype (CC, CT or TT) and in non-infectious diseases [23].

The membrane CD14 receptor of macrophages plays a key role in the inherited immune response. It is a multipotential receptor that binds bacterial components and supports signalling pathways of Toll-like receptors. The role of the sCD14 receptor in development of human diseases has not yet been clarified. However, increase of sCD14 levels in the course of various diseases is believed to result from activation of monocytes and macrophages, which throw off a membrane form of this receptor from their surfaces. Also it cannot be excluded that elevation of circulating sCD14 levels remains the essential mechanism of removal of excess of bacterial proinflammatory factors, including LPS. Noteworthy sCD14 mediates LPS activation of cells without their own membrane CD14 receptor, such as endothelial cells, which may be important in the context of progression of ischaemic heart disease.

In conclusion, the outcomes of this study do not allow screening tests for 159C/T polymorphism of the CD14 receptor gene to be recognised as a method of identification of patients at risk of MI at younger age, regardless of lack of traditional risk factors of

atherosclerosis, in a population with high cardiovascular morbidity. However, together with studies on sCD14 and LBP, they considerably improve our understanding of the role of the inflammatory reaction in the pathogenesis of IHD. It seems there is a need to continue current studies in larger populations of patients and healthy volunteers. Such studies should focus not only on the importance of ethnic aspects, necessity of selection of appropriate group sizes, simultaneous participation of various genes in atherogenicity, but also on the possibility of modification of genetic features by cultural and social factors as well as medical interventions. This was also brought up by other investigators [24].

Conclusion

Genetic screening for 159C/T polymorphism of the promoter region of the CD14 receptor gene in patients with CAD from a population with high cardiovascular morbidity and mortality is unlikely to be a useful tool in identifying patients at risk of MI at younger age and with low number of traditional risk factors, despite the fact that distribution of the genetic variants differs from other ethnic groups.

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Polimorfizm 159C/T genu receptora CD14 w grupie pacjentów z chorobą wieńcową z populacji o wysokim wskaźniku chorobowości z powodu chorób układu krążenia

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Streszczenie

Wstęp: Rola receptora CD14 w reakcjach zapalnych i aterogenezie polega na pośredniczeniu w stymulacji monocytów, komórek śródbłonka naczyń i komórek mięśni gładkich przez lipopolisacharyd bakterii Gram-ujemnych takich drobnoustrojów, jak kojarzone z rozwojem miażdżycy *Chlamydia pneumoniae* czy *Helicobacter pylori*. Wyniki badań nad polimorfizmem regionu promotorowego genu receptora CD14 i jego związkiem z rozwojem i destabilizacją blaszek miażdżycowych są sprzeczne, gdy badani pochodzą z różnych etnicznie grup.

Cel: Ocena, czy polimorfizm regionu promotorowego genu CD14 159C/T (zmiana nukleotydu C na T w pozycji 159) wiąże się z wiekiem wystąpienia pierwszego zawału mięśnia sercowego (MI), stopniem zaawansowania zmian miażdżycowych w naczyniach wieńcowych przy pierwszej w życiu koronarografii lub z liczbą skumulowanych klasycznych czynników ryzyka dla choroby niedokrwiennej serca w populacji charakteryzującej się wysokim wskaźnikiem chorobowości na choroby układu krążenia (województwo łódzkie), a także czy przesiewowe badania genetyczne ukierunkowane na poznanie wariantów genetycznych w pozycji 159 promotorowego regionu genu receptora CD14 mogą być pomocne w zidentyfikowaniu w tej populacji osób zagrożonych MI w młodym wieku, niezależnie od braku tradycyjnych czynników ryzyka.

Metodyka: Grupę 57 chorych po przebytym MI, z potwierdzoną koronarograficznie chorobą wieńcową (43 mężczyzn, 75,5%) wyselekcjonowano z 89 kolejnych chorych leczonych w 2003 r. w Klinice Kardiologii Uniwersytetu Medycznego w Łodzi. Układ nukleotydów w pozycji 159 dla genu CD14 zbadano za pomocą techniki polimerazowej reakcji łańcuchowej (PCR). Profil kliniczny pacjentów, w tym: obecność cukrzycy, nadciśnienia tętniczego, dyslipidemii, palenia tytoniu, otyłości, określono na podstawie historii chorób. Na podstawie wyniku badania koronarograficznego obliczono także wskaźnik *Gensini score* (GS) jako liczbową miarę nasilenia miażdżycy tętnic wieńcowych. Użyte metody statystyczne to: test t-Studenta dla porównania wartości średnich zmiennych; test χ^2 – dla porównania rozkładów wartości i test u – dla porównania dwóch częstości występowania danej cechy.

Wyniki: Genotyp CC stwierdzono u 25 (43,8%) chorych, CT u 30 (52,6%), a TT u 2 (3,6%). Wobec niewielkiego odsetka chorych z genotypem TT, wariant ten analizowano łącznie z wariantem CT. Średni wiek wystąpienia pierwszego MI wynosił 58,7±7,23 lat (40–75. roku życia). Średnia wartość GS – 48,6±25,7 (0–154). Liczba czynników ryzyka: średnio 1,92±0,99 (0–4). Nie stwierdzono istotnych statystycznie różnic pomiędzy pacjentami z genotypem CC a pacjentami z genotypem CT lub TT w zakresie średniej wieku wystąpienia pierwszego MI – odpowiednio 58,7 i 60,6 lat ($p > 0,05$), ani odsetka osób, u których zawał serca wystąpił przed 50. lub po 70. roku życia ($p > 0,05$). Nasilenie miażdżycy wyrażone wg GS także nie różniło się istotnie porównywanych grup: średnia wartość GS dla wariantu CC wynosiła 49,2, a dla CT lub TT – 48,1 ($p > 0,05$). Także średnia liczba czynników ryzyka miażdżycy (odpowiednio 1,88 i 1,95) ani odsetek chorych z < 2 czynnikami ryzyka (36 i 31%) nie różniły się istotnie statystycznie ($p > 0,05$).

Wnioski: Pomimo specyficznego rozkładu poszczególnych wariantów nukleotydów w pozycji 159 regionu promotorowego genu receptora CD14, różnego od rozkładu w innych populacjach (rzadkie występowanie genotypu TT) – badania przesiewowe tego genu nie wydają się użytecznym narzędziem identyfikującym w badanej populacji osoby zagrożone wystąpieniem MI w młodym wieku, niezależnie od braku lub niewielkiej liczby czynników ryzyka.

Słowa kluczowe: polimorfizm CD14, zawał serca, badania przesiewowe

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