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RESEARCH

Tumornecrosis-Factor-α 308 GA Polymorphism in Atherosclerotic Patients

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Abstract The development of the atherosclerosis is a multifactorial process, where the clinical pattern is determined by environmental and genetic factors. Except for the classical risk factors of atherosclerosis (hypertension, lipid-metabolic disorders, diabetes, smoking) the clinical signs can be influenced by the genetic variants (polymorphisms) of the enzymes, which are responsible for the endothelial cell function and for the thrombotic factors. In our examination our aim was to define the TNF-α 308GA polymorphisms in atherosclerotic diabetic, atherosclerotic non-diabetic and healthy patients. We found correlation of the frequency of myocardial infarction and stroke in atherosclerotic diabetic and atherosclerotic non-diabetic patients. We proved that among patients with mutant TNF-α AA genotype the occurrence of cardiovascular events is significantly higher: Mutant AA homozygous genotype: control group 1, 6%, MI group 10,7%, $p<0,005$, OR: 8,17 versus Normal GG allele: control group 76,7%, MI group 61,3%. The TNF-α AA genotype can have a clinical importance as a prognostic and therapeutic marker, although further studies are needed to confirm this hypothesis.

Keywords Tumornecrosis-factor-α · Polymorphism · Atherosclerosis · Cardiovascular risk

Abbreviations

TNF-α	Tumornecrosis-factor-α
DM	Diabetes mellitus
MI	Myocardial infarction
ASO	Atherosclerosis obliterans

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Introduction

In the last decade it was confirmed, that inflammation has great importance in the development of atherosclerosis [1]. Earlier the atherom was thought to be a simple laesion, now it is cleared, that the local inflammation and immune response play role in the atherogenesis.

A close connection was found between the high level of CRP and the endothelial dysfunction [2, 3] and many paper proved the key-role of the tumornecrosis factor as a trigger in the endothelial dysfunction [4, 5]. In the atherosclerotic plaque many cellular and molecular processes indicate that as very similar to the chronic inflammation. It is confirmed, that the level of inflammation-related plasma-proteins in the cardiovascular diseases elevates. These transmembran proteins seem to play a role in the cell-cell interaction [6], they have metalloprotease sequence on their extracellular part, which makes them able to activate the TNF-α [7]. The ineffective function of the vascular endothelial cells has a great importance also in the plaque-evolution [8]. The result of abnormal endothelial cell function is the release of inflammation cytokines, like interleukin-1b, tumornecrosis factor-α and C-reactive protein [9]. The inflammatory process results high level of CRP that will activate the monocytes and macrophages. The TNF-α is one of the most important mediator of the inflammatory answer [5, 10, 11]. The TNF-α has importance in the pathophysiology of vascular diseases, through the lipid metabolism, obesity and influences the insulin resistance. Nowadays obesity itself can be a critical risk factor, because lipid tissue produces TNF-α, which affects on the vascular wall directly or influences the insulin metabolism [10, 12, 13]. In the development of the metabolic syndrome the cytokines, especially the tumornecrosis factor-α were found to have a great importance [14].

It is cleared that the elevated TNF-α level has role in the development of the myocardial ischemia and infarction [4,

15]. Due to the progression of the atherosclerotic plaque the fibrotic cap increases, on the surface the enzyme activity (like TNF-α) is getting higher, so the thrombocyta adhesion will be intensified, thrombus can develop.

Since the genetical factors temper with the TNF-α expression level, it is important to know its polymorphisms, can be found in its promoter region.

In this study our aim was to determine the TNF-α 308GA polymorphism in atherosclerotic non-diabetic, atherosclerotic diabetic patients and in healthy control samples. We found a connection between the occurrence of TNF-α 308GA polymorphism and the diabetes, atherosclerosis related stroke and MI.

Materials and Methods

In the examination data of 992 people's were inspected, 608 of them were treated on the Cardiovascular Department of the Semmelweis University. A 250 of them TNF-α polymorphism were checked. All clinical and laboratorial data were recorded. They all underwent different vascular surgical treatments, and received the compulsory thrombocyte antiaggregation treatment and statins. The patient group was divided into two groups: one group had both the atherosclerosis and diabetes, the other group had not such metabolical disease. MI and stroke can be mentioned as the endpoint of the atherosclerosis, we observed its frequency in the different groups. Four subgroups were formed, such as a./ diabetes + MI, b./ diabetes + stroke, c./ atherosclerosis + MI, d./ atherosclerosis + stroke. The control sample comes from the National Institute of Oncology they were all healthy people, who had undergone a complete screening examination.

The data were statistically analysed by a two-side Student t-probe, Odds ratio counting, Fischer's exact test, chi-square-probe, average and scatter measurement (SD), graphical presentment. Those difference were considered as significant, where the statistical deviance were *p<0,05, and **p<0,005.

DNA Isolation

For the DNA isolation 3–6 ml of periferial blood in EDTA-containing tubes was used. It was kept on −20°C until the separation. For the isolation Wizard Genomic DNA Purification Kit (Promega) was used.

Genotype and SNP Analysis

For the TNF-α 308GA polymorphism determination the following PCR was used:

5′-AGGCAATAGGTTGAGGGCCAT-3′ and 5′-TCCTCCCTGCTCCGATTCCG-3′

The 308GA polymorphism determination was performed due to F. Real method [16].

Results

Regarding ages there was no great difference between the groups. The mean age was 70 years in the atherosclerotic diabetic and 68 years in the atherosclerotic non-diabetic group. The youngest patient was 40, the oldest 93 years old. Half of the diabetic patients got per os medication (NIDDM), one third of them received insulin (IDDM), the others followed a diet.

The frequency of MI was similar in the two main groups, 38,9% in the atherosclerotic diabetic and 39% in the atherosclerotic non-diabetic group. The stroke prevalence was much higher in the atherosclerotic diabetic group (38% versus 20%).

Table 1 TNF-α gene polymorphism frequency atherosclerotic diabetic and atherosclerotic non-diabetic patient group, combined with MI or Stroke

Study group	TNF-α Homozygote Mutant 308 AA %	TNF-α Heterozygote Mutant 308 GA %	TNF-α Homozygote Normal 308 GG %
1. Control (n=184)	1,6	21,7	76,7
	3/184	40/184	141/184
2. ASO + DM+ MI (n=65)	9,2 *	26,2	64,6
	6/65	17/65	42/65
3. ASO + DM + Stroke (n=65)	7,7**	30,7	61,6
	5/65	20/65	40/65
4. ASO + MI (n=75)	10,7**	28	61,3
	8/75	21/75	46/75
5. ASO + Stroke (n=45)	11,1*	28,9	60,0
	5/45	13/45	27/45

ASO artherosclerosis obliterans, *DM* diabetes mellitus, *MI* myocardial infarction

*p<0,05, **p<0,005 vs. control

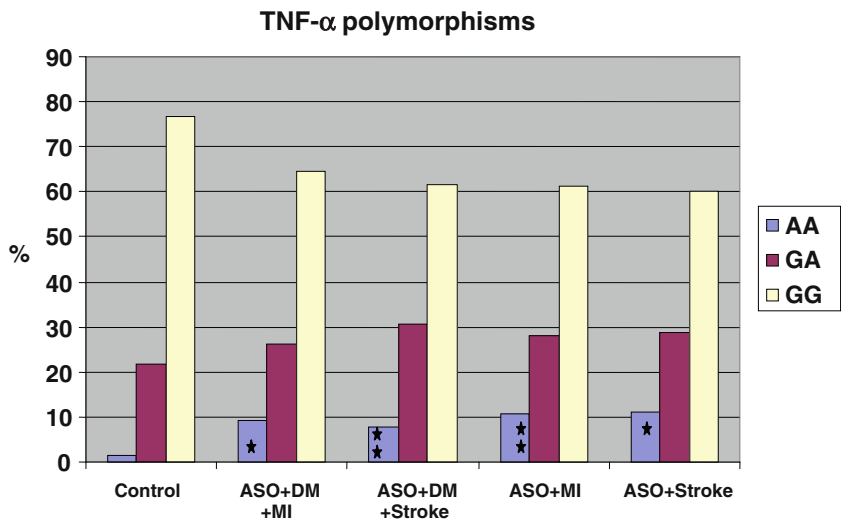

Fig. 1 The frequency of the TNF-α polymorphisms in the five examined groups. The prevalence of the mutant 308 AA homozygote genotype in the atherosclerotic non-diabetic MI and in the atherosclerotic diabetic stroke group is significantly higher to the control group (*p<0,05, **p<0,005). (*DM* diabetes mellitus, *MI* myocardial infarction, *ASO* atherosclerosis obliterans, *AA* homozygote mutant allele, *GA* heterozygote mutant allele, *GG* homozygote normal allele)

We examined the frequency of different TNF-α polymorphisms in various illness-combinations. The 308 mutant homozygote AA, the normal homozygote GG and the heterozygote GA prevalence were checked in case of MI and stroke in the atherosclerotic diabetic and atherosclerotic non-diabetic patient groups (Table 1).

Our present findings suggest that the prevalence of mutant AA genotype in the control group is low, 1,6%, since in the diabetic + MI group it is 7,7% (p<0,005). In atherosclerotic + MI group 10,7%, (p<0,005), in the atherosclerosis + stroke group 11,1% and that is also significant compared to the control group (p<0,005). The frequency of heterozygote GA genotype does not deviate significantly in the relation of the control (Fig. 1).

At the Odds ratio measurement we found that in the case of homozygote mutant AA genotype the frequency of all type diseases is significantly high. In the atherosclerotic-diabetic group the MI risk is high, OR 6,71 (1,72–25,55), even the stroke is elevated, OR 5,87 (1,48–23,21). In the atherosclerotic non-diabetic group the risk of MI is high, also, OR 8,17 (2,24–29,55), but the stroke risk is the highest OR 8,71 (2,15–34,98).

Discussion

The development of the atherosclerosis is a multifactorial process, where the clinical pattern is determined by environmental and genetic factors. Except for the classical risk factors of the atherosclerosis (hypertension, lipid-metabolic disorders, diabetes, smoking) the clinical signs can be influenced by the genetic variants (polymorphisms) of the enzymes which are responsible for the endothelial cells function and for the thrombotic factors. It is cleared, that the diabetes elevates the risk for cardiovascular diseases. There are several facts which ensure that the inflammatory cytokines play a key role in the process [17].

In an earlier study we examined the importance of eNOS 298GA (nitrogen oxide synthase) and MTHFR 677CT (methylentetrahydrofolate reductase) polymorphisms in

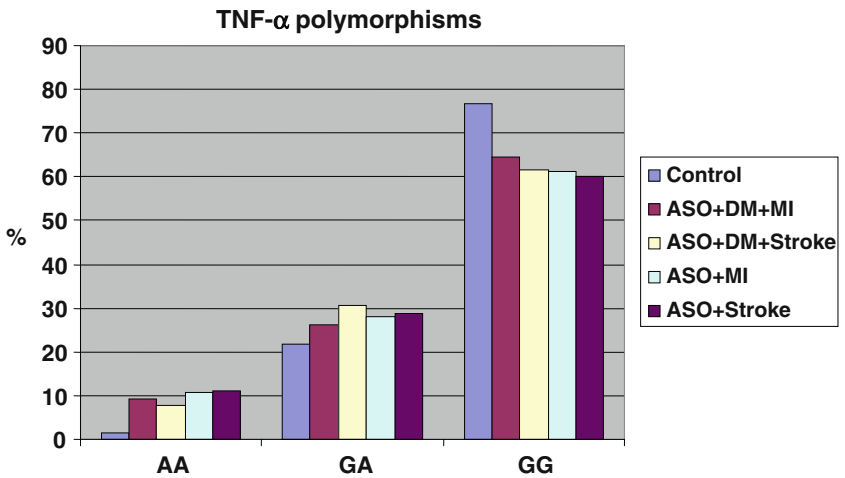

Fig. 2 The correlation of the TNF-α polymorphisms in healthy control, atherosclerotic diabetic, atherosclerotic non-diabetic patient groups, made the groups by alleles. In the patients groups the mutant AA allele elevation was the highest in percentage in comparison with the controls. (*DM* diabetes mellitus, *MI* myocardial infarction, *ASO* atherosclerosis obliterans, *AA* homozygote mutant allele, *GA* heterozygote mutant allele, *GG* homozygote normal allele)

several patient groups. The higher risk affected those patients, who were Asp298 homozygote (TT), they had four times higher risk for cardiovascular diseases than those who were Glu298 homozygote (GG). In the relation of the MTHFR we proved that even the heterozygote CT and also the homozygote mutant TT allele caused significantly, three times higher cardiovascular risk compared to the control healthy group [18].

Among the cytokines the tumornecrosis factor plays a key role. Simvastatin and atorvastatin, which are used to cure hyperlipidemia and hypercholesterinemia reduce the level of TNF-α [19]. TNF-α takes part in the mechanism of vasodilatation, through the synthesis of the free radicals and the nitrogen oxide (NO) level control [20]. It has a great importance in the development of cardiovascular diseases. The expression level of the TNF-α is influenced by the 308GA polymorphism, located in the promoter region of its gene. In the case of the mutant 308 AA homozygote and 308 GA heterozygote allele the TNF-α effect is elevated.

In this study we investigated 184 healthy controls, 65 atherosclerotic diabetic and 75 atherosclerotic non-diabetic samples. We analysed TNF-α 308 GA polymorphism in different illness combination. We established, that in healthy control population the wild-type TNF-α 308 GG allele occurrence is 76,7%, the heterozygote 308 GA is 21,7%, while the 308 mutant AA is 1,6%.

We established, that at atherosclerotic diabetic patient with MI (2. examination group) the 308 AA mutant allele occurrence is elevated to 9,2% compared to the control group 1,6% ($p<0,05$). In the 3. examination group (DM+ Stroke) the mutant AA allele prevalence is 7,7% (against the control 1,6% it is significantly high, $p<0,005$), while the GA heterozygote is 26,2%, the wild GG is 64,6%.

Those patients, who carried the A allele, had a highest TNF-α level, that increased their risk for cardiovascular diseases [21].

In the 4. and 5. examination group there were the atherosclerotic non-diabetic patients. The 4. group with MI, in the 5. group with stroke. The high-risk-causing AA allele occurs significantly more frequently (10,7%, 11,1%), than in the control group (1,6%) ($p<0,005$). The occurance of heterozygote 308 GA allele is also higher (28%, 28,9%), than the occurrence in control (21,7%) but it is not significant.

Earlier studies showed that in France and North-Ireland's population the TNF-α 308AA genotype occurred often in patient with myocardial infarction [22]. Other studies verified correlation between the TNF-α level and the atherosclerosis only at young patients [23]. Our graphic shows the different genotype prevalence among average Hungarian population (Fig. 2).

The TNF-α seems to have an important role in the development of the stenosis of the arteries [11]. Its affects the atherosclerotical process in several points. Blocking its biosynthesis the plaque evolution and the development of neointima will be slower [11, 13, 19]. Different polymorphisms of this gene have different cardiovascular risk—the highest is in the case of AA mutant allele.

Screening the endangered or genetically high risk groups is to be considered on the long run—an early detection of a susceptibility of the disease gives better chances for prevention and treatment. Understanding the inflammatory mechanisms of the atherosclerosis gives new therapeutical targets to pharmacologists.

References

1. Yang Z, Ming X (2006) Recent advances in understanding endothelial dysfunction in atherosclerosis. Clin Med Res 4 (1):53–65
2. Blanke G, Ridker P (2001) Inflammatory mechanisms in atherosclerosis: from laboratory evidence to clinical application. Ital Heart J 2(11):796–800
3. Ablij HC, Meinders AE (2002) C-reactive protein: history and revival. Eur J Int Med 13:412–422
4. Ridker P, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E (2000) Elevation of tumor necrosis factor-alfa, and increased risk of recurrent coronary events after myocardial infarction. Circulation 101:2149–2153
5. Stenvinkel P (2001) Endothelial dysfunction and inflammation—is there a link? Nephrol Dial Transplant 16:1968–1971
6. Shah P (1998) Role of infalammation and metalloproteinases in plaque disruption and thrombosis. Vasc Med 3:199–206
7. Geisler T, Bhatt DL (2004) The role of inflammation in atherosclerosis: current and future strategies of medical treatment. Med Sci Monit 10(12):308–316
8. Stoll G, Bendzus M (2006) Inflammation and atherosclerosis: novel insights into plaque formation and destabilization. Stroke 37:1923–1932
9. Fiotti N, Giansante C, Ponte E, Delbello C, Calabrese S, Zacchi T, Dobrina A, Guarnieri G (1999) Atherosclerosis and inflammation. Patterns of cytokine regulation in patients with periferial arterial disease. Atherosclerosis 145:51–60
10. Libby P, Ridker PM, Maseri A (2002) Inflammation and Atherosclerosis. Circulation 105:1135–1143
11. Monraats PS, Pires NMM, Schepers A, Agema W, Boesten L, Vries M, Zwinderman A, de Maat M, Doevendans P, Winter R, Tio R, Waltenberger J, Hart L, Frants R, Quax P, Vlijmen B, Havekes L, Laarse A, Wall E, Jukema W (2005) Tumor necrosis factor- α plays and important role in restenosis development The. FASEB J 19:1998–2004
12. Dalziel B, Gosby A, Richman R, Bryson J, Caterson I (2002) Association of the TNF-α (−308 G/A) promoter polymorphism with insulin resistance in obesity. Obes Res 10(5):401–407
13. Hotamisligil G (2006) Inflammation and metabolic disorders. Nature 444(14):860–867
14. Nieszner É, Bárdos P, Baranyi É, Préda I (2005) A diabetes mellitus cardiovascularis szövődményei és diagnózisuk. LAM 15 (10):722–729
15. Vendrell J, Fernandez-Real JM, Gutierrez C, Gutierrez C, Zamora A, Simon I, Bardaji A, Ricart W, Richart C (2003) A polymorphysm in the promoter of the tumor necrosis facrtor-α gene (−308) is associated with coronary heart disease in type 2 diabetic patients. Atherosclerosis 167:257–264

16. Fernandez-Real JM, Gutierrez C, Ricart W, Casamitjana R, Fernandez-Castaner M, Vendrell J, Richart C, Soler J, 9 (1997) The TNF-alpha gene Nco I polymorphism influences the relationship among insulin resistance, percent body fat, and increased serum leptin levels. Diabetes 46(9):2096–2098
17. Welt FGP, Rogers C (2002) Inflammation and restenosis in the stent era. Arterioscler Thromb Vasc Biol 22:1769–1776
18. Szabó GV, Kunstár A (2009) Acsády Gy.: methylentetrahydrofolate reductase and nitric oxid synthase polymorphysm in patients with atherosclerosis and diabetes. Pathol Oncol Res 15:631–637
19. Ascer E, Bertolami M, Venturinelli M, Buccheri V, Souza J, Nicolau J, Ramires JA, Serrano C (2004) Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. Atherosclerosis 177:161–166
20. Goodwin BL, Pendleton LC, Levy MM, Solomonson LP, Eichler DC (2007) Tumor necrosis factor- α reduces argininosuccinate synthase expression and nitrix oxide production in aortic endothelial cells. Am J Physiol Heart Circ Physiol 293:1115–1121
21. Wilson AG, Symons JA, McDowell TL, McDevitt H (1997) Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation. Proc Natl Acad Sci USA 94:3195–3199
22. Herrmann SM, Ricard S, Nicaud V, Arveiler D, Evans A, Ruidavets JB, Luc G, Bara L, Parra HJ, Poirier O, Cambien F (1988) Polymorphisms of the tumour necrosis factir- α gene, coronary heart diseases and obesity. Eur J Clin Invest 92:14–8
23. Elkind MS, Cheng J, Boden-Albala B, Rundek T, Thomas J, Chen H, Rabbani LE, Sacco RL (2002) Tumor necrosis factor receptor levels are associated with carotid atherosclerosis. Stroke 33:31–38