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MARKER TNF -308G/A IN PATIENTS WITH RELATIVE BLADDER CANCER

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Abstract:

The aim of the study was to determine the TNF- gene polymorphism in the blood of patients with stage T1N0M0 bladder cancer and to establish their relationship with the grade G malignancy in case of disease recurrence. The study included data from 130 patients with stage T1NoMo bladder cancer: with disease recurrence in 66 patients (main group) and 64 patients with bladder cancer without disease recurrence (control group), including 75 men and 55 women.

Keywords: TNF -308G/A MARKER , Relative Red Cancer

INTRODUCTION: According to the World Health Organization, bladder cancer ranks 7th in the structure of oncopathology in men and 17th in women. More than 380 thousand new cases of bladder cancer are diagnosed annually. This disease causes the death of 150 thousand people, while the sex ratio is 3.8: 1. In recent years, the incidence of bladder cancer has been steadily increasing in Uzbekistan. In 2005, 997 patients were registered for bladder cancer, in 2001 - 1078, and at the end of 2018 there were already 1401 patients. The incidence of newly diagnosed disease also increases from 201 cases in 1995 to 280 in 2000 and to 303 cases in 2017 [1, 2, 7]. Urothelial cancers are a common pathology in the bladder, but their karyotypic characteristics and genetic pathway remain poorly understood. Most of the data presented are quantitatively limited and do not have karyotypic accuracy [6,9]. Tumor necrosis factor- (TNF-) is an inflammatory cytokine, mainly produced by macrophages, that can induce cell growth, death and regeneration. The gene encoding TNF- has achromosomal locus of 6p21.3, and there are several polymorphisms upstream of the proximal TNF- promoter that can affect its production and biological activity. Recently, several studies investigating the relationship between TNF-polymorphism and genitourinary cancer risk have been performed, yielding results that suggest that TNF- polymorphism 308G / A [3,12] TNF- is mainly produced by activated macrophages, and two TNF- gene polymorphisms have been found to increase the risk of bladder cancer [5,11]. This could potentially be due to TNF- induced regulation of thymidine phosphorylase, an enzyme that has been shown to contribute to the development of bladder cancer. TNF- also promotes angiogenesis and the development of several types of tumors. The TNF- genes are located on chromosome 6 in the major histocompatibility complex (MHC) region adjacent to the lymphotoxin locus. Sixteen gene polymorphisms have been identified, two of which are TNF + 488A and TNF- 859T. The TNF + 488A polymorphism is found in 28.1% of bladder cancers and 14.9% of healthy people, while TNF-859T is present in 26.6% of bladder cancers and only 14.4% of healthy patients. The role of these various polymorphisms in cancer is not well understood due to conflicting results from various studies [4,8,10]

The aim of the study: to determine the TNF- gene polymorphism in the blood of patients with stage T1N0M0 bladder cancer and to establish their relationship with the G grade in case of disease recurrence.

MATERIALS AND METHODS OF RESEARCH

The study included data from 130 patients with stage T1NoMo bladder cancer: with disease recurrence in 66 patients (main group) and 64 patients with bladder cancer without disease recurrence (control group), including 75 men and 55 women. The average a - data of 64 patients without disease recurrence were used as a control. The material for DNA isolation was venous blood from the cubital vein with a volume of 3-5 ml (Beckton-Dickinson vacutainers were used for blood sampling) with an anticoagulant / preservative 15% tripotassium EDTA (Ethendianin-tetraacetic acid). Blood for further processing could be stored for up to 24 hours at a temperature not exceeding two-stage blood cell lysis method was used. Lysis of erythrocytes was carried out by double centrifugation of the entire volume of

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whole blood in RCLB (Red cells lysis buffer) at a speed of 1500 rpm for 15-20 minutes. The use of RCLB induces an osmotic shock of erythrocytes, leading to their swelling and further destruction. The supernatant containing destroyed erythrocytes was carefully decanted from the tube, and the remainder of the supernatant was aspirated. The clot of the leukocyte mixture remaining at the bottom was lysed in the leukocyte lysis buffer WCLB (White cells lysis buffer) in an amount depending on the volume of the leukocyte mixture. WCLB is also a preservative for the storage of leukocyte lysates even at room temperature. In this state, the lysates could be stored indefinitely. Further purification of leukocyte mass lysates is based on the method of alcohol-salt treatment according to S. Miller et al. (1988) in the modification proposed by the Stanford University laboratory. To 400 l of leukocyte mass lysates, 150 l of 5M NaCl was added, mixed on a shaker and placed on ice for 10-20 minutes, then centrifuged at 1200 rpm for 15 minutes. The supernatant was taken into the another Eppendorf tube and 100% ice-cold ethanol was added. With gentle shaking, a quaternary chain of the DNA molecule appears in the mixture; the mixture was centrifuged at 1200 rpm for 15 minutes; the supernatant was removed, and the whitish spot remaining on the bottom of the tube was washed again in 80% ethanol at 1200 rpm for 10 minutes. The supernatant was discarded, the residual alcohol was carefully removed, the tube was left open until the alcohol completely evaporated (for 12 hours at room temperature or in a thermostat at 40- After the alcohol was evaporated, a TE (Tris-EDTA) solution diluted with distilled water was added to a test tube with dried DNA in a ratio of 1: 3 (TE: water) pH 8.0. Genotyping of polymorphic regions of the TNF gene (G-308A) was carried out by polymerase chain reaction (PCR) with allele-specific primers (NPF Litekh, Moscow) and electrophoretic detection of reaction products in agarose gel, these SNPs are previously confirmed and have a minor allele frequency 1% or more. (NCBI dbSNP database) [6,8]. The distribution of genotypes at the studied polymorphic loci was studied using logistic regression analysis and checking for compliance with the Hardy Weinberg equilibrium using Fisher's exact test. The correspondence of patients and persons of the control group by sex and age was taken into account. Differences were considered statistically significant at $p < 0.05$. The identification of amplification products and their distribution in relation to the length marker was carried out in ultraviolet light (310 nm) after electrophoresis for 15 minutes or in 10% PAGE 29: 1 at a voltage of 300 V (in both cases, the run was 3-4 cm) and staining ethidium bromide. A digest of plasmid pUC19 with restriction enzyme Msp1 was used as a length marker. The analysis of the obtained data was carried out using standard methods of statistical processing using the Microsoft Office Excel software and the Statistica v6.0 software package. Statistical processing of the obtained data was carried out by methods of nonparametric statistics. The nonparametric Mann-Whitney U-test and Wilcoxon test were used as a criterion for the significance of the difference between the groups. Also, the obtained data are presented in the form of a median with a minimum and maximum run-up for nonparametric components. 95%. CI (confidence interval) was estimated using random and fixed effects models [7,11]

RESULTS AND THEIR DISCUSSION

A comparative study of the frequency distribution of alleles and genotypes of polymorphic markers of the TNF -308G / A gene in groups of patients and in the control (Table 1) revealed a statistically significant increase in the frequency of the A allele in patients compared with the control group (9.38% and 3.72 %, respectively; OR = 2.675; 95% CI: 1.023> 2.675> 6.992; 2 = 4.304 (p = 0.03802)). At the same time, the G allele of the studied polymorphism was much less common compared to the control group (90.63% and 96.28%, respectively; OR = 0.374; 95% CI: 0.143> 0.374> 0.977; 2 = 4.304 (p = 0.03802)) . Further, a comparative analysis of TNF -308G / A genotypes for the GG genotype revealed significant differences between the patients and the control group (81.25% and 92.55%, respectively; OR = 0.349; 95% CI: 0.129> 0.349> 0.942; 2 = 4.598 (p = 0.032003)). Analysis of the heterozygous GA genotype revealed differences between the frequency of occurrence in patients and the control group (18.75% and 7.45%, respectively; OR = 2.868; 95% CI: 1.062> 2.868> 7.745; 2 = 4.598 (p = 0.032003)). As already described above, a significant difference was found in the frequency of occurrence of the A allele, the studied TNF -308G / A polymorphism, but the genotypic analysis of the homozygous AA genotype was not detected. Overall, a significant association was found between TNF- 308G / A polymorphism and risk of urogenital cancer (A versus G: OR = 1.18, 95% CI = 1.06-1.32, P = 0.002; GA versus GG: OR = 1.19, 95% CI = 0.3-1.37, P = 0.012; GA / AA versus GG: OR = 1.20, 95% CI = 1.07-1.36, P = 0.003) (Table 2). For urothelial carcinoma, the OR (95% CI) was 3.56 (1.03 12.29) in the homozygous model and 3.52 (1.02 12.13) in the recessive model. These results show that is, significant associations between TNF-polymorphism 308G / A urothelial carcinoma with and without relapse TNF- level in patients with bladder

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cancer stage T1N0M0. According to the study, in patients with bladder cancer of stage T1N0M0, the average level of TNF- erence was not statistically significant (p> 0.05). In patients with bladder cancer and the degree of neoplasia G1, the average level of TNF-degree G2 - neoplasia G3 - 40.01

CONCLUSIONS

Our study regarding the determination of the TNF- marker in the blood of patients with stage T1N0M0 bladder cancer has established that the level of malignancy G has a prognostic parameter in terms of recurrence of the disease, while it is necessary to clarify that the TNF- 308G gene polymorphism has a colossal role in the increase in the level of malignancy. / A, while this difference was statistically significant. The data obtained indicate that the -308 (G / A) TNF polymorphism contributes to the predisposition to disease recurrence at genetic levels and is one of the prognostic factors in patients with bladder cancer. In conclusion, we would like to say that the TNF- 308G / A polymorphism and the risk of recurrence of urogenital cancer are largely interrelated.

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