GENETIC POLYMORPHISMS OF TOLL-LIKE RECEPTORS AT TRIPLE-NEGATIVE BREAST CANCER

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Breast cancer is the most common cancer among women in Ukraine [1]. Approximately from 8% to 20% are categorized as triple-negative breast cancer (TNBC) that does not overexpress a human epidermal growth factor receptor 2 (HER-2/neu), estrogen receptor (ER) and progesterone receptor (PR) [2]. Recent experiments have shown that toll-like receptors (TLRs) can enhance cancer cell progression, induce evasion of immune surveillance, and induce chemotherapeutic and metastasis [3]. TLRs polymorphisms may be involved in the process of breast carcinogenesis and play an important role in cancer development and treatment.

The aim of this study was to investigate *TLR2*(G753A), *TLR4*(C399T), *TLR9*(G2848A) polymorphisms and analyze their association with TNBC development.

In the present study, samples of peripheral blood of 63 patients with TNBC from age 24 to 76 years (52 ± 3.5 years) were used as a biological material to investigate polymorphisms of *TLR2, 4* and *9* genes. After DNA extraction, we used polymerase chain reaction (PCR) method to make many copies of the *TLR* 2, 4 and 9 genes with specific primers. The PCR products of *TLR2* were digested by restriction endonuclease SsiI (AciI) at 37 ºC overnight and then analyzed by 3% agarose gel electrophoresis. Bands of 228, 75 and 40 bp corresponded to TLR2 G/G; 268, 228, 75, 40 bp bands were designated as heterozygous G/A individuals; a band of 268, 75 bp corresponded to the homozygous A/A genotype. The PCR products of *TLR4* were digested by restriction endonuclease Hinf I. It was incubated overnight at 37ºC and electrophoresed in 3% agarose gel to identify the TLR4 alleles on the basis of the respective allele size. Bands of 406 bp corresponded to *TLR4* C/C; 406, 377, 29 bp bands were designated as heterozygous C/T individuals; a band of 377, 29 bp corresponded to the homozygous T/T genotype. The PCR products of *TLR9* were digested by restriction endonuclease Bsh 1236 I (BstU I) at 37 ºC overnight and then analyzed by 3% agarose gel electrophoresis. Bands of 135, 42 bp corresponded to *TLR9* G/G; 177, 135, 42 bp bands were designated as heterozygous G/A individuals; a band of 177 bp corresponded to the homozygous A/A genotype.

According to the results, in the group of 56 patients with TNBC, were found 76.8% (43/56 patients) – with homozygous genotype of the wild type *TLR2* allele (genotype G/G), 5.4% (3/56 patients) – with homozygous genotype of the mutant type allele (genotype A/A) and 17.9% (10/56 patients) with heterozygous genotype (genotype A/G). Patients with the A/A genotype of the *TLR2* gene have significant increased risk (in 13.15 times) of TNBC development compared to the control ($\chi 2 = 11.49$; $p = 0.003$).

Our results indicated that *TLR4*(C399T) is not associated with risk of TNBC development, as all of the 60 patients had CC genotype - homozygous genotype of the wild type allele of the *TLR4* gene.

According to the results, in the group of 59 patients with TNBC, were found 33.9% (9/59 patients) – with homozygous genotype of the wild type *TLR9* allele (genotype G/G), 15.3% (20/59 patients) – with homozygous genotype of the mutant type allele (genotype A/A) and 30.5% (30/59 patients) with heterozygous genotype (genotype A/G). Patients with the A/A genotype of the *TLR9* gene have significant increased risk (in 9.77 times) of TNBC development compared to the control ($\chi 2 = 95.94$; $p = \leq 0.0001$).

We established that presence of *TLR2*(G753A) and *TLR9*(G2848A) polymorphisms have an increased risk of TNBC development and may be recommended to the diagnostic marker in the primary screening of BC. Our results showed that *TLR4*(C399T) is not associated with the TNBC development.

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