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ARTICLE

Detection of glutathione S-transferases M1, T1 gene deletions among cancer patients in Mongolia

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Abstract: *Various types of toxic xenobiotic and electrophilic compounds, which were formed from the glutathione S-transferases cell metabolism and the oxidation stress, are the group enzymes with detoxification roles that are involved in the metabolism phase II. During the GSTM1 and GSTT1 gene homozygous deletion, the above enzymes completely lose their activity and consequently somatic mutation is formed. Furthermore, it is considered that it might have increased the risk of cancer. Therefore, the research works which connected the GSTMI and GSTTI gene deletion with the cancer of kidney, lung, prostate, breast, stomach, esophagus, large and narrow intestines. In this study, two gene deletion distribution is detected for cancer patients. We collected the blood samples of 60 patients who have been diagnosed with cancer. The DNA was extracted and the GSTM1 and GSTT1 genes were amplified using multiplex PCR. According to our research, the above two gene deletion is predominant among patients who have cancer. The results showed that from the total 60 patients GSTM1 and GSTT1 both deletions, GSTM1 gene deletion - 35%, GSTM1 gene deletion - 25%, GSTT1 gene deletion - 26.7%, GSTM1 and GSTT1 both positive -13.3 %. Therefore, we think that in order to prevent tumor and cancer, these gene mutations must be revealed and it is important to bring the risky group under medical control and assist them in order to prevent them from this disease.*

Keywords: *GSTM1; GSTT1; polymorphism; cancer;*

INTRODUCTION

The number of the patients in Mongolia who have been diagnosed with cancer has been increasing from year to year and approximately about 4,000 people have died of cancer. People in Ulaanbaatar city and the rest of the country are living in quite a risky condition in as much as medicinal drug use is inappropriate and often times without prescription, and consume food with high level of food preservatives, which are tolerant to pesticides and herbicides etc. Food safety is not guaranteed in addition to high level of air and environment pollution.

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One of the distinctive features of the human body is that it has the system to render harmless external-source toxic or dangerous combinations which are formed as a result of metabolism. One of them is GST or glutathione S-transferases, which are a family of ubiquitously expressed polymorphic enzymes that are involved in the metabolism phase II [13][9][7][5]. The main biological role of Glutathione S-transferases is to protect the cell membrane and DNA from damage owing to regular metabolism oxidation and detoxifying products, given that there are so many types of carcinogen and electrophilic compounds (such as ketones, quinones, sulfoxides, esters, peroxides, and ozonides), chemotherapeutics (such as busulfan, cis-platin, ethacrynic acid, cyclophosphamide, thiotepa) industrial chemicals herbicides, pesticides (acrolein, lindane, malathion, tridiphane) that are involved, directly and indirectly to render them harmless. Glutathione S-transferases is genetically determined as polymorphic. It is considered that several of the GST polymorphic participate in cancer development, medicine haul against cancer, and it influences its metabolism and also participate in the other diseases [2] [4][5][7][10][11][12] [14] [16].

The GST that exists in the human cell's cytoplasm is divided into 8 classifications. From them, isoforms of GSTM1, GSTT1, and GSTP1 have been most commonly studied by researches. For the genotype, the GSTM1, GSTT1 become null. Therefore, the particular gene does not have the coded enzyme and thus becomes inactive or non-binding, which is why it is studied and researched with great attention [4][5][14][15]. In this study, we have tried to determine how the distribution of two gene deletion is detected.

MATERIALS AND METHODS

Samples: were taken from the outpatient clinic of the Mongolian National Cancer Center. Sample blood came from 60 patients who were diagnosed with cancer diagnosed, who had undergone surgical operations and those who were undergoing chemical and radiation treatment at the center's out-patient clinic. Genomic DNA was isolated using the chloroform method.

Apex. 2.0XTaq Red (1.5mM MgC12) Master mix kit was used. The reaction's total volume – 20ul, 10ul of master mix, 1ul of each primer, 1.5ul of extracted DNA as template for gene amplification. The GSTM1 primers, (f: 5'- GAA CTC CCT GAA AAG C-3', r: 5'- GTT GGG CTC AAA TAT ACG GTG G-3') were used for the amplification of the 218 bp fragment. The primers of GSTT1 F: 5'- TTCCTTACTGGTCCTCACATCTC3'. R: 5'- TCACCGGATCATGGCCAGCA-3'-were used for the amplification of the 480bp fragment. The cycling condition involved an initial pre-denaturation at 94^0C for 4 minutes, and followed by 32 cycles of PCR amplification. Amplification conditions were as follows: denaturation at 95^0C for 1 minute, primer annealing at 58^0C for 1 minute, and extension at 72^0C for 10 minutes. The final extension step at 72^0C for 6 minutes ended the process with the help of Arktik^{TM} Thermal Cycler., Thermo fisher was increased. PCR amplicons were separated on 1.6% agarose gel and stained with ethidium bromide. Bands were visualized under UV light and GSTM1, GSTT1 bands were detected with the presence of bands 218bp and 480bp on the gel. The patients were grouped as either positive or null genotypes.

RESULTS AND DISCUSSION

For patients who have GSTT1, GSTM1 and GSTP1 gene changes, have high probability of having cancers of the bladder, breast, large and narrow intestines, brain and lung. Therefore, in our study we involved 60 cancer patients - 25 of them had liver cancer, 8 - breast cancer, 5 - ovarium cancer, 5 - cervix of uterus cancer, 4 - rectum cancer, 4 - stomach cancer. Involved in our study were also cancer patients who had oral, lung, testicle, thyroid,

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esophagus and prostate cancer. The sex ratio was 41 - female and 19 - male. In terms of age, they were aged between 32 and 76 years old.

Another study, Ashton et al., 2007, found that GSTM1 null genotype is associated with high risk of neuroblastoma in Australia and New Zealand. It has been observed through research that children with GSTT1 gene deletion and AML-disease are more likely to die after chemical treatment [5]. Also in another research, during both the GSTT1, GSTM1 gene deletion, people who have the AML-disease have higher risk of becoming sick again after the treatment [4]. These gene deletion contribute to increase in rapture, allergy, atherosclerosis, and also partially prompts rheumatoid arthritis risk, therefore utmost care must be taken. According to published data, genetic diversity in the Japanese population shows slightly higher GSTM1 null genotype (55.8%) and significantly higher GSTT1 null genotype (40.0%) frequencies. The GSTM1 and GSTT1 genotype frequencies of the control population in our study were in accordance with other reported frequencies. There is yet another research which revealed that 75% (highest) of the Caucasian citizens have the GSTM1 gene deletion [16] from other genotype. According in this study, which covered a total of 60 patients, GSTM1 and GSTT1 both deletions and GSTM1 gene deletion was 35%, GSTM1 gene deletion was 25%, GSTT1 gene deletion was 26.7%, GSTM1 and GSTT1 both without deletion was 13.3 %.

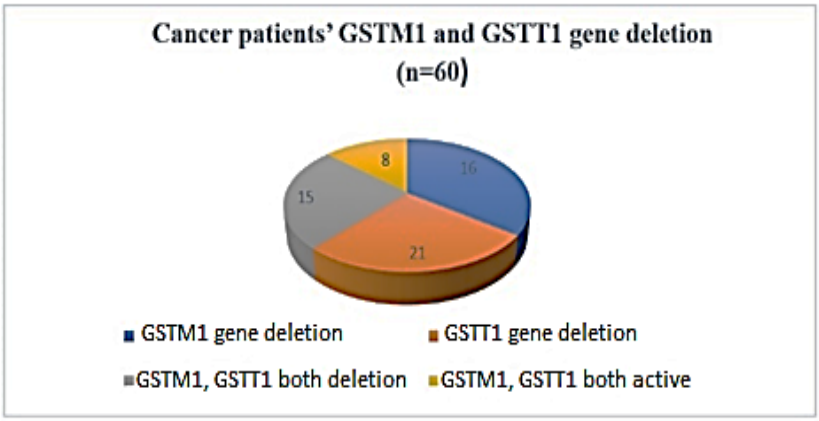

Figure 1. GSTM1 and GSTT1 gene deletion indication among cancer patients

In the event if there is both GSTM1 and GSTT1 gene deletion, and when either one of them is compared, then GSTM1 and GSTT1 gene deletion was higher for patients aged 32 to 50. In our research, it was observed that there are quite a substantial number of cancer patients in Mongolia, have both the GSTM1 and GSTT1 gene deletion. Either GSTM1 and GSTT1 gene without-deletion or positive version was revealed in 8 patients, who had ovarium, thyroid, rectum and liver tumor.

When considering a combination of alleles of the GSTT1 and GSTM1 genes, the proportion of deletated alleles of both genes in patients was slightly higher (35%) than in the separate GSTT1 - 25%, GSTM1 - 26.6% (Table1), whereas the frequency of both alleles without deletions is 13.3% (Figure 3).

Table 1. Distribution of GSTM1, GSTT1 genotypes in the study groups

Gene	Deletion	
	%	n
GSTM1	26.6	16
GSTT1	35	21
GSTM1 GSTT1	25	15

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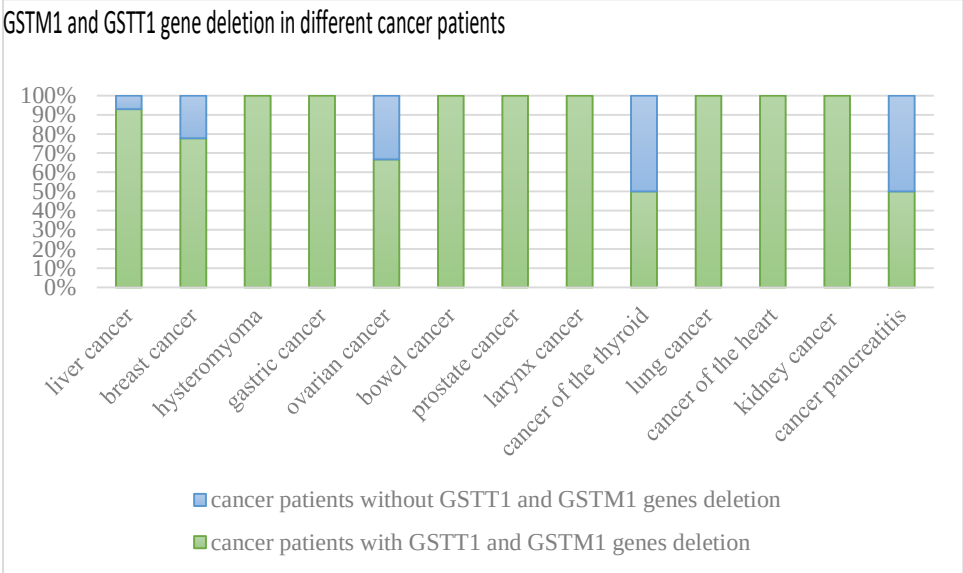

Figure 3. GSTM1 and GSTT1 gene deletion in different cancer patients

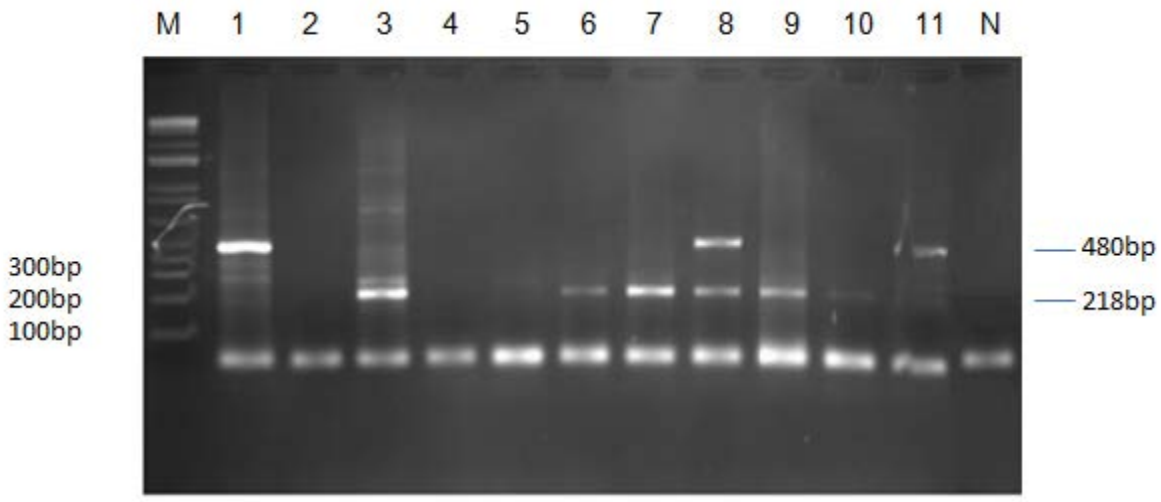

Figure 4: Multiplex PCR result (with number 1,8,11 – GSTT1/480bp/ positive., 3,6,7,8,9,10 numbers – GSTM1 /218/ positive., 2,4,5 – GSTM1, GSTT1 gene both have the deletion, number 8 – GSTM1 and GSTT gene both positive, N - negative control)

A different multiple case-controlled studies revealed statistically significant associations of the GSTM1 null genotype with increased risk of childhood cancers.

Research groups from different regions in the world found that GSTM1 null genotype is related to increased risk of acute leukemia (ALL and ALP) in various populations.

CONCLUSIONS

Cancer is a multifactorial disease, one of which is hereditary predisposition and the surrounding environment. Our work for the first time shows that the increased risk of cancer among Mongolian cancer patients may be associated with the zero genotype of the GSTM1, GSTT1 genes. According to our research, the above two gene deletion is predominant among patients who have fallen ill dues to cancer (86.6%). Therefore, we think that in order to prevent from tumor and cancer, these gene mutation needs to be revealed and the risk groups must be taken under medical control and they must be given assistance in

Vol. 59 No 04 (232) 2019
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preventing this disease.

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