# Genetic Polymorphisms of Glutathione S–transferase M1 and T1 and the Risk of Hepatocellular Carcinoma*

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**Abstract** **Objective** To study the genetic polymorphisms of Glutathione S–transferases and their relation to hepatocellular carcinoma (HCC) at an aflatoxin high contaminated area in Guangxi, China. **Methods** Polymerase chain reaction (PCR) technique was used to detect the presence or absence of the GSTM1 and GSTT1 genes in the blood samples of 110 patients of HCC which were identified by histopathologist, and the control group was composed of 135 adults without any tumor. **Results** The frequencies of GSTM1 and GSTT1 null genotype in the control were 47.4% and 40.7% respectively, while in the HCC group were 63.6% and 60.4% respectively. The differences between the two groups were significant, $P<0.05$ (M1) and $P<0.01$ (T1). **Conclusion** Both GSTM1 or/and GSTT1 null genotypes are common in natives population in this AFB1 high contaminated area of Guangxi. It is the risk factors for the natives predisposed to HCC. Genetic susceptible plus environmental factors caused the high prevalent of HCC.

**Key Words** hepatocellular carcinoma; aflatoxin B1; Glutathione S–transferase

Aflatoxin B1 (AFB1) is a mycotoxin that produced by aspergillus flavus. It is a vigorous carcinogen and has a liking act on hepatocytes[1]. It has been found that AFB1 contaminated food such as corn and peanut in the world, and it is known that southwest of Guangxi is one of the area with rather high level of contamination and concomitant with high prevalent of hepatocellular carcinoma (HCC)[2–3]. AFB1 is not harmful prior to metabolic activation via oxidase P450 to form AFB1–8, 9–epoxide[4] , the latter is a mutagenic product act on DNA. There are also several enzymes in the body to resist this toxin and good for health. The present study emphasize the importance of polymorphisms of glutathione S–transferase M1 and T1 (GSTM1 and GSTT1), especially their genetic deletion polymorphisms and susceptible to HCC.

## *MATERIALS AND METHODS*

**Patients and controls** 110 HCC patients and 135 controls participated in the present investigation. The HCC group cases were recruited patients from affiliated hospital of Guangxi Medical University, from January 1998 to December 2001. The patients include 92 males and 18 females, aged from 28 to 70 with an average of 47.5 years old. All their HCCs had been confirmed by pathologic diagnosis, The control group came from the same hospital health check up adult persons excluding the tumor cases.

**Blood Samples** 5ml of blood were taken by venous puncture. The blood was used for lymphocytes isolation with the standard erythrocyte lysis procedure. Genomic DNA was prepared by standard phenol–chloroform extraction.

**PCR** Primers were designed to detect GSTM1 (273 bp) and GSTT1 (480 bp)[5–6]. Primers for the GSTM1 were 5′–CTGCCCTACTTGATTG–ATGGG–3′ and 5′–CTGAATTGTAGC–AGATCATGC–3′. Primers for the GSTT1 were 5′–TTCCTTACTGGTCCTCACATCTC–3′ and 5′–TCACCGGATCATGGCCAGCA–3′.

PCR reaction was carried out in a "BIO–RAD" amplified instrument. A commercial PCR kit was used with 5μg of DNA, 2.5m Mol/L of dNTP, 5u Mol/L of each primer, 25m Mol/L of $MgCl_2$ and 0.5 units of Taq polymerase in a total volume of

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25μl, then overlaid with a drop of mineral oil and proceed to amplification.

**PCR amplified condition** The samples were pretreatment at 94℃ for 5 min, then treated in a different way for GSTM1 and GSTT1 genes amplification. Followed by 30 second at 93℃, 45 second at 50℃ and 72℃ for 45 second for the amplification of GSTM1 gene, and 45 second at 50℃, 50 second at 61℃ and 72℃ for 60 second for the GSTT1 gene, respectively. After 35 cycles circulated, all followed by a final step of extension at 72℃ for 10 min. The amplified products were subjected to electrophoresis on a 2% agarose gel and stain with ethidium bromide, observed under violet ray instruments and noted down the results and taken photographs. In the cases of GSTM1 or GSTT1 gene deletion, the samples must be internal control by a pair of β-globin primer co-amplification to exclude the possible pseudo-negative reaction owing to condition not fit[6].

**Statistical analysis** The experimental results were calculated by $\chi^2$ test.

## *RESULTS*

PCR products from co-amplification of GSTM1 (480 bp) and GSTT1 (215 bp) on agarose gel are shown in Fig.1 and Fig.2.

The rate of GSTM1 null genotype in HCC group and control group were 63.6% and 47.4% respectively. The difference is significant (P<0.05). The rate of GSTT1 null genotype in HCC group and control group was 60.0% and 40.7% respectively, the difference is very significant ($P$<0.01) (table 1). The rate of both GSTM1 and GSTT1 being null genotype in HCC group and control group are 38.2% and 18.5% respectively, the difference is significant ($P$<0.05). In situation of GSTM1 positive but GSTT1 Negative, case group versus control group are 21.8% versus 20.7%; and in the reverse situation of GSTM1 negative but GSTT1 positive, the case group versus control group are 25.5% versus 28.9%; all the latter two situations are no significant difference (table 2).

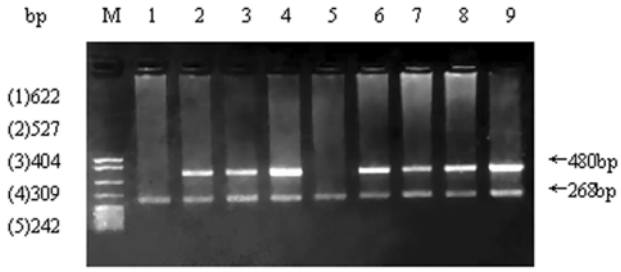


Fig.1 Co-amplification products of GSTT1 gene and β-globin on agarose gel
GSTT1 480bp, β-globin 268bp.
M: pBR322 DNA/Msp I, Lane1: control group GSTT1 deficiency,
5:HCC group GSTT1 deficiency, 2-4:control group GSTT1 normal,
6-9 HCC group GSTT1 normal, 1-9: β-globin

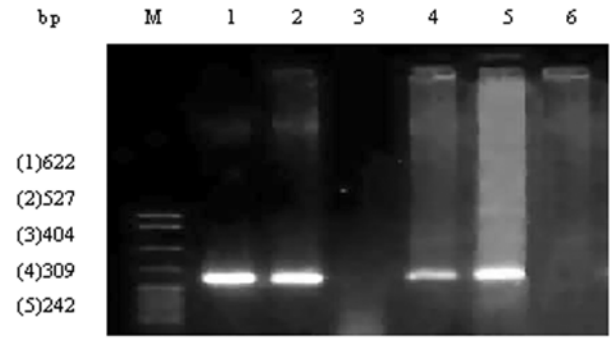


Fig.2 Amplification products of GSTM1 gene electrophoresis on agarose gel
GSTM1 273bp.
M: pBR322 DNA/Msp I, Lane-2: HCC group GSTM1 normal,
4-5: control group GSTM1 normal, 3: HCC group GSTM1 deficiency,
6:control group GSTM1 deficiency

## *DISCUSSION*

Glutathione S-transferase (GST) plays a major role in the detoxification of many toxic compounds in human body. GST gene is a super-family, seven classes had been found, but only class mu (GSTM1) and class theta (GSTT1) were shown to have gene deficiency (null genotype)[7-8] and com-

Table 1 Polymorphisms of GSTM1 and GSTT1 gene between HCC patients and controls

| Group | N | GSTM1 | | | GSTT1 | | |
|---|---|---|---|---|---|---|---|
| | | Null | Present | Deletion rate(%) | Null | Present | Deletionrate(%) |
| HCC | 110 | 70 | 40 | 63.6 | 66 | 44 | 60.0 |
| Control | 135 | 64 | 71 | 47.4* | 55 | 80 | 40.7** |

The HCC group compare with control * $\chi^2$ = 6.4424, P<0.05 ** $\chi^2$ = 8.9942, $P$<0.01

Table 2 Polymorphisms of GSTM1 and GSTT1 genes and risk of HCC

| GST | GSTM1-(%)* | | GSTM1+(%)** | |
|---|---|---|---|---|
| | HCC | Control | HCC | Control |
| GSTT1- | 42(38.2) | 25(18.5) | 24(21.8) | 28(20.7) |
| GSTT1+ | 28(25.5) | 39(28.9) | 16(14.5) | 43(30.4) |

*$\chi^2$ =5.8630, $P$=0.015, OR=2.340, 95%Cl=1.170~4.681
**$\chi^2$ =4.3449, $P$=0.037, OR=2.304, 95%Cl=1.044~5.084

pletely lack of relevant enzyme activity. Since carcinogens of AFB1-8, 9-epoxide are substrates of both GSTM1 and GSTT1[9-10]. The absence of these enzymes may be susceptible to AFB1 carcinogen. We already have known that AFB1 is one of the carcinogens that play a major role in the local area high prevalence of HCC [2]. It is need to know whether natives of Guangxi susceptible to HCC and whether HCC patients posses more GSTM1 or GSTT1 null genotype than other people[11-12,17].

Salagovic et al (1998, 1999) reported that smokers with GSTM1 and GSTT1 null genotypes were susceptible to the lung and urinary bladder carcinomas [13-14]. Cheng et al reported that smokers with GSTM1 and GSTT1 gene deficiency predisposed to head and neck squamous cell carcinoma[15]. Kim et al (2000) indicated that young women(under age 40) with GSTM1 and GSTT1 null genotypes increased the risk of cervical carcinoma [16]. This study showed that local area in southwest of Guangxi where natives have rather higher level of GSTM1 and GSTT1 null genotypes. Herein GSTM1 null genotype of natives is 47.4% that is a high level in the world. While GSTT1 null genotype is 40.7%, which is much higher than the average level. It is known that people with genetic high GST null genotype but they never contact with relevant chemical toxin such as AFB1 and they will never increase the risk of HCC. However, long span of time living in an AFB1 contaminated area will have enough time for fully revealed its genetic defect and susceptible to HCC. The present study showed that GSTM1 or GSTT1 null genotypes cases are higher in the HCC group than in control group, the differences are significant, ($P$<0.05 and $P$<0.01 respectively). There is no any relationship to their age and sex status ($P$>0.05). The frequency of both GSTM1 and GSTT1 concomitant deletion in HCC group and control group are 38.2% and 18.5% respectively, the difference is significant ($P$<0.05). Actually, there is twice the risk of HCC in the concomitant null genotype cases than control. In conclusion, the present study found that the natives of Guangxi are in high level of GSTM1 or/ and GSTT1 null genotype that is a genetic defect. Natives in the southwest of Guangxi unfortunately meet the situation of the food contamination by AFB1. Genetic factors plus environmental factors cause high prevalent of HCC. Especially in the case of both genes deficient cases they are even more susceptible to HCC[17].

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