



Genetic Analysis of Restriction Fragment Length Polymorphism of TLL1 Gene (rs17047200) in Patients of Hepatocellular Carcinoma

Saima Sharif¹, Sadia Ashraf¹, Shagufta Naz¹, Sher Zaman Safi², and Muhammad Saqib³

¹Department of Zoology, Lahore College for Women University, Lahore,

²Interdisciplinary Research Centre in Biomedical Materials, COMSATS University, Lahore

³District Head Quarter Hospital, Nankana Sahib, Sheikhpura

Abstract: To find out the association of polymorphism of the TLL1 gene in hepatocellular carcinoma. A cross-sectional study was conducted from January 2020 to September 2020. Subjects were enrolled from Mayo Hospital, Lahore, Jinnah Hospital Lahore and the Liver Transplant Unit of Sheikh Zayed Hospital, Lahore. A total of 200 individuals were registered and segregated into the Control group (n=100) and hepatocellular carcinoma (HCC) group (n=100). DNA was extracted from obtained blood samples and Restriction Fragments Length Polymorphism (RFLP) was carried out at the laboratories of LCWU by using specific primers and restriction endonuclease enzymes. The data were analyzed statistically. The high proportion of smoking, hepatitis B, hepatitis C, cirrhosis and Body Mass Index (BMI) were established risk factors in the HCC group. Subjects with hepatocellular carcinoma had low socioeconomic status. Heterozygous bands in the HCC group were observed after RFLP. TLL1 genotype was AA (72 %) and AT/TT (28 %). The patient's clinical aspects were similar across TLL1 genotypes. It was concluded that RFLP on the exon region by using their specific enzymes HpyCH4III showed heterozygous bands in the HCC group that indicated a mutation in the TLL1 gene though this mutation does have a significant association with HCC.

Keywords: Liver Cancer, Polymorphism, Hepatocellular Carcinoma, PCR, TLL1 gene

1. INTRODUCTION

Globally, the prevalence of liver cancer is increasing which is also acknowledged as primary hepatic cancer or primary hepatic malignancy. Hepatocellular carcinoma (HCC) includes about 80 percent of intrahepatic cholangiocarcinoma. In addition, HCC is linked to abdominal mass and pain, anemia, itching, fever, back pain, weight loss, and jaundice [1]. Moreover, it is more common in men as compared to women [1]. In the past few years, the landscape of hepatocellular carcinoma (HCC) has been rapidly growing and also accounts for approximately 75 percent of all primary liver malignancies [2]. Cancer-related death was caused due to HCC. The incidence of Hepatocellular carcinoma (HCC) shows substantial global variation which is dependent on the differences in HCC risk factors including exposure to co-carcinogens as well as viral hepatitis. The highest

incidence of Hepatocellular carcinoma had shown in sub-Saharan Africa and Southeast Asia [1, 3, 4]. the incidence and cancer mortality is increasing in the developing world. Similarly, Pakistan faces disturbing limitations in cancer care that have an adverse impact on patient outcomes [5].

Numerous risk factors include smoking, hepatitis B virus, cirrhosis, metabolic disease, genetically liver disorders, diabetes, lack of antioxidant vitamins and selenium, non-alcoholic fatty liver disease, long-term excessive drinking, intake of aflatoxin, hepatitis C virus, in women with a long-term oral contraceptive, chronic and inorganic lead poisoning, and iron-overload are the prime peril for the hepatic tumor. Inclusion of contaminants and exposure to risk factors proves to be the reason for the appalling liver cancer. Hereditary vulnerability of hepatic tumors becomes the main focus of research [6, 7].

Mammalian tolloid-like 1 is zinc-dependent matrix metalloproteases which are also known as TLLI and belong to a subfamily known as the bone morphogenetic protein 1 as BMP1 as well tolloid-like proteinases (BTPs). BMP1 is the first discovered tolloid of the family in mammals and is also known as procollagen C-endopeptidase, which involves the formation of the extracellular matrix. In humans, Tolloid-like protein 1 is encoded on the TLL1 gene located on chromosome 4 [8]. In hepatocarcinogenesis after hepatitis c virus elimination or liver fibrosis in a patient with nonalcoholic fatty liver disease, the single nucleotide polymorphism in Tolloid-like 1 and expression of TLL1 were closely related [9].

In our study, we aimed to analyze the genetic factors in the Pakistani population and the role of polymorphism of TLL-1 (rs17047200) in association with liver cancer. To the best of our knowledge, this is the first study for genetic analysis of RFLP of the TLL1 gene (rs 17047200) in Pakistani subjects of HCC.

2. METHODOLOGY

The study was conducted in different hospitals including Mayo Hospital, Jinnah Hospital and Sheik Zayed Hospital, Lahore in Lahore from January 2020 to September 2020. Patients who have Hepatocellular Carcinoma (HCC) were recognized after they were clinically examined by the doctors. Healthy subjects were also enrolled as a control group and both groups were ethnically matched. The Ethical Committee of LCWU and hospitals approved the study. Information of the enrolled subjects regarding age, sex, family history, weight, height, body mass index (BMI) the antecedent of other cancers, carcinogenic treatments, contraindication related to surgery, pregnancy-related issues, embryo-procure tumor, agile liver ailment, and the other factors were collected through a self-designed questionnaire.

2.1 Criteria Used

For study subjects the inclusion and exclusion criteria were as follow:

2.1.1 Inclusion Criteria

Subjects who were suffering from liver cancer

like Hepatocellular Carcinoma, as well as fibrosis together with liver cirrhosis, were taken into consideration in this study, and subjects without any disease were referred to as a control group.

2.1.2 Exclusion Criteria

Subjects who have any other disease such as cardiovascular disease or kidney disease were excluded from this study as well as from the control group.

Before the collection of blood samples, the informed consent form was taken from each subject. The collected blood samples were stored at -20 °C and DNA was separated by following the procedure of Gimberg *et al.* (1989) [10]. The polymerase chain reaction-restriction length polymorphism (PCR-RFLP) technique was adopted to analyze the TLL1 gene polymorphisms using specific primers as shown in Table 1. Gel electrophoresis was carried out to see the products of enzyme digestion.

Table 1. The primers of the TLLI gene

S. No.	Gene	Primer	GC Content	Product Size
1	TLL1 (R)	5' CCA TAG GAA GCA ATG CTG	47.62	492bp
		AAC 3' 5' CTG TTG		
2	TLL1 (F)	ACC TAA GAC GTA ATG G-3'	45.45	

2.2 Statistical Analysis

All statistical analysis was carried out using Microsoft Excel and Statistical data for Social Sciences (SPSS, version 19.0), and the t-test was used to compare the means of two groups.

3. RESULTS

The Clinical Attributes of the study groups were presented in Table 2.

Molecular analysis of the TLL1 gene was conducted using Polymerase Chain Reaction (PCR) restriction fragments length polymorphism (RFLP) in both the control and HCC groups. After DNA amplification of the exon VII region by using its

respective primers, we got the PCR products of 492bp when compared with the ladder as shown in figure 1 & figure 2.

Restriction Fragments Length Polymorphism (RFLP) was performed to analyze if any polymorphism of the TTL-1 gene was present. RFLP was performed on the exon region by using their specific enzyme HpyCH4III. The Heterozygous

bands in the HCC group obtained through RFLP indicated mutation. The homozygous bands in the control batch had appeared. TLL1 genotype was AA (72 %) and AT/TT (28 %). The patient's clinical aspects were similar across TLL1 genotypes. In the HCC group, the heterozygous bands appeared which shows the association of the TTL-1 gene with hepatocellular carcinoma.

Table 2. Demographic data of the studied population

Variables	Control group	HCC group
Age (years)	29.8 ± 5.11	38.3 ± 7.14**
Weight (kg)	59.5 ± 9.79	61.3 ± 6.61**
Height (cm)	5.26± 0.54	5.20 ± 0.58
BMI (kg/m ²)	24.4 ± 5.79	26.14 ± 6.61*

*Significant variation between groups (p≤0.05).

**Highly notable difference among the groups (p≤0.01)

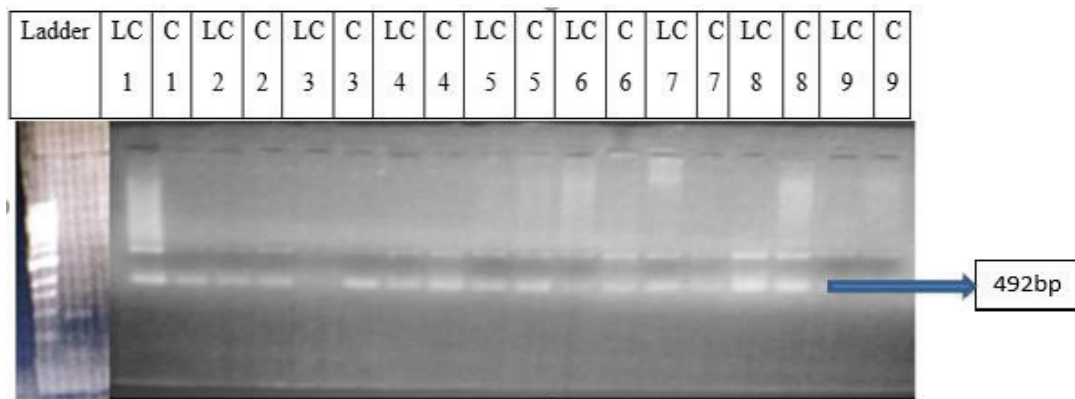


Fig. 1. PCR product obtained by using specific primers



Fig. 2. RFLP performed for the digestion of amplified PCR products.

4. DISCUSSION

HCC is not only the sixth most widespread category of cancer in the world but also the third uncouth reason for cancer deaths [11, 12]. The prevalence of hepatocellular carcinoma was high in males (80 %) than the females (20 %) ($p \leq 0.01$). The current study depicts that the prevalence of hepatocellular carcinoma is high in males as compared to females. A similar result has been reported by the American cancer society, (2016) that the probability of liver cancer significantly soars high in males as compared to females. Obesity is a major complication. A report by the world health organization report describes that obesity all over the world has been two-fold since 1980. Our study depicts that in the hepatocellular carcinoma group 65 % of individuals were found overweight. It has been found that there is more prevalence of overweight in the HCC group compared with the control group.

It has been assessed in another study that the risk of developing liver cancer is increased due to being obese which could be resulted in cirrhosis and fatty liver disease [1].

Smoking is one of the leading sources of hepatocellular carcinoma and is also the major cause of many diseases. In the present study, the percentage of smokers was high in the HCC group in comparison with the control group. In the present study, in the hepatoma group, the pervasiveness of current and former smokers was 25 % and 65 % respectively. It has been reported in [13] that smoking increases the chances of liver cell cancer. In the previous studies, it was stated that lower risk had been found in former smokers as compared to current smokers, but both groups have a higher risk than those who never smoked [14].

Cirrhosis is a hazardous quotient for the process of hepatic cell carcinoma. The danger is 3–4 times elevated in persons with cirrhosis than in the ones with dreadful hepatitis in a given population. An increase in hepatocellular proliferation may cause enlivening of oncogenes and mutation of tumor suppressor genes. Changes like these may initiate hepatic carcinogenesis [15].

In low-incidence areas, cirrhosis had been

reported in more than 90 % of patients with hepatocellular carcinoma. However, in high-incidence areas, the presence of cirrhosis was less (approximately 80 %), in these areas the vertical transmission of hepatitis B virus was common. Whereas, in this study cirrhosis was also the reason for hepatocellular carcinoma.

There are many genetic factors associated with the process as well as the occurrence of tumors which are very complicated and include genomic instability, protooncogene activation, epigenetic alteration, inactivation of the antioncogene, chromosome gain and deletion, and epigenetic alteration [16].

In this study, it was reported that the variant was eliminated with the specific fast reactive enzyme HpyCH4III on the restriction site of PCR products. The same size of heterozygous bands appeared in the affected individuals as in the control group. It was predicted that the GLT-1 allele depicted a greater frequency in HCC persons compared to controls. The small size of the sample made us unable to chalk out a stronger link in the familial HCC group. It was also investigated that the features of other genes, genes and their environmental interactions, epigenetic elements have not been calculated, which may be the staggering variables. Additionally, Matsuura *et al.* study reported the progression of fibrosis in liver tissue patients that had increased Levels of TLL1 mRNA. The patients had higher rs17047200 AT/TT with gene expression levels of TLL1 short variants, such as isoform 2 [17].

Furthermore, Hong *et al.* (2015) stated that more knowledge could be obtained about the pathogenesis of liver cancer by exploring the susceptibility genes. However, the is not dependent on a single gene. It could be caused due to interactions of mutations in various genes [16].

In this study, we were not able to confirm the significant association between TLL1 genotypes and clinical features of liver cancer patients. Moreover, TLL1 genotypes did not influence HCC features at diagnosis. Several other factors may potentially explain the differences between associations of genotypes in the TLL1 gene with clinical features.

However, it requires further studies that may pay attention to gene-environment interaction as well as gene-gene interactions. So, a better understanding and deepened knowledge of liver cancer may be obtained.

5. CONCLUSION

RFLP on the exon region by using their specific enzymes HpyCH4III showed heterozygous bands in the HCC group that indicated a mutation in the TLL1 gene but the studied genotype was not associated with HCC. So some other factors may potentially affect differences between associations of genotypes in the TLL1 gene with clinical features.

6. CONFLICT OF INTEREST

The authors declared no conflict of interest.

7. ETHICAL APPROVAL

This study was conducted with approval from the Ethics Committee of the Hospitals.

8. PATIENT'S CONSENT

A document of informed consent had signed by all patients.

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