Association between MDM2-SNP309 polymorphism and hepatocellular carcinoma in Egyptian patients with chronic viral hepatitis

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Noha Kamal

TABLE OF CONTENTS

List of abbreviation:

A2M: Alpha-2-Macroglobulin
AAR(AST/ALT): Aspartate aminotransaminase/ Alanine aminotransaminase ratio
ADAMTS: interlukin A Disintegrin And Metalloproteinase with Thrombospondin Motifs
AFP: Alpha (α) fetoprotein
AFU: Serum alpha-L-fucosidase
ALP: Alkaline phosphatase
ALT: Alanine aminotransaminase
ANA: Anti-neutrophil antibody
ANG: Angiogenin
Anti-HCV: antibody to hepatitis C virus
APO: apolipoprotein
APOE: apolipoprotein E
APRI: AST to platelet ratio index
ARF: alternate reading frame
ARF: ADP ribosylation factor
ASMA: Anti–smooth muscle antibody
AST: Aspartate aminotransaminase
AUROC: The area under the receiver operating characteristic curve
BASP1: Brain acid soluble protein 1
bDNA: branched DNA .
BIRC1: Baculovirus Inhibitor of apoptosis protein Repeat containing proteins 1
CBC: complete blood picture
CCNG1: Cyclin-G1
CCR5: C-C chemokine receptor 5
CD44: cluster differentiation 44
COL1-A1: collagens collagen, type I, alpha 1
CRS: cirrhosis risk score
CT: Computed tomography
CTBP1: C-terminal-binding protein 1
CTBP2: C-terminal-binding protein 2
CTGF : Connective tissue growth factor
CTLA4: cytotoxic T-lymphocyte-associated protein 4
CXCL9 and CXCL10: chemokines C-X-C motif ligant 9 and 10
CXCR3: C-X-C motif receptor 3
CYP2C8: Cytochrome $P_{450}2C8$
D.Bil.: Direct bilirubin
DCP: Desgamma-carboxy prothrombin
ECD: Extracytoplasmic Domain
ECM: Extracellular matrix
EF: Early fibrosis
EGFR: epidermal growth factor receptor
EGR-1: Early growth response-1
EIA: enzyme immune assay
ELF: Enhanced liver fibrosis
ELISA: enzyme-linked immunosorbent assay
ENPP1/ PC-1: ectoenzyme nucleotide pyrophosphate phosphodiesterase 1/ plasma cell antigen-1
EP300: E1A binding protein p300

ET 1: endothelin 1
FKBP3: FK506-binding protein 3
FOXO4: Forkhead box protein O4
GGT II: Serum gamma-glutamyltransferase isoenzyme II
GGT: Gamma-glutamyl transferase
GJB2: Gap junction beta-2 protein
GNL3: Guanine nucleotide-binding protein-like 3
GPC3: Glypican-3
GS domain: rich in glycine and serine residues
HA: Hyaluronic acid
Hb: Haemoglobin
HbeAg: Hepatitis B e antigen
HbsAg: Hepatitis B surface antigen
HBV: Hepatitis B virus
HBX: HBV protein X
HCC: Hepatocellular carcinoma
HCCR: Human cervical cancer oncogene
HCV: Hepatitis C virus
HDAC1: Histone deacetylase 1
HFE: high iron Fe
HHT: Hereditary hemorrhagic telangiectasia
HIF1A: Hypoxia-inducible factor 1, alpha subunit
HMEC-1: Human umbilical vein endothelial cells-1
HPA: human platelet antigens
HSC: Hepatic stellate cells
HSP70:70 kilodalton heat shock proteins
HTATIP: Histone acetyltransferase Tat-interactive protein
ICAM1: Intercellular Adhesion Molecule 1
ICAM2: Intercellular adhesion molecule 2
IFNγ: interferonγ
IGF2: Insulin-like growth factor 2
IGF–II: serum insulin-like growth factor-II
IGFs/IRS/MAPK: insulin growth factors / Insulin receptor substrate / Mitogen-activated protein kinases
IL-10: Interleukin-10
IL-18: interleukin-18
IL-2R: Inteleukin-2 receptors
INR: International Normalised Ratio
IRB: Institutional Review Board
IRS-1: insulin receptor substrate-1
ITIH1: inter-alpha-trypsin inhibitor heavy chain 1
LAMB1: Laminin subunit beta-1
LDLR: low density lipoprotein receptor
LYVE: lymphatic vessel endothelial hyaluronan receptor
MDM4: murine double minute 4
MF: Myofibroblast
MGB: minor groove binder
MKI67: monoclonal antibody Ki-67
MMPs: Matrix metalloproteinases
MPO: myeloperoxidase

MRI: Magnetic resonance imaging
MTP: metalloproteinase
NAFLD: Nonalcoholic fatty liver disease
NASH: Non-alcoholic steatohepatitis
NC1:Carboxyterminal cross-linking domain
NO: Nitric oxide
PBC: Primary biliary cirrhosis
PC: Prothrombin concentration
PCAF: P300/CBP-associated factor
PCR: polymerase chain reaction,
PDGF: Platelet derived growth factor
PICP: Procollagen type I carboxy terminal peptide
PIIINP: Procollagen III amino peptide
PIVKA-II: Protein induced by vitamin K absence/antagonist-II
PIVNP: aminoterminal 7S domain of procollagen type IV
PLG: plasminogen
PSC: Primary sclerosing cholangitis
PSME3: Proteasome activator complex subunit 3
PT: Prothrombin time
RBCs: Red blood cells
ROC: Receiver operating characteristics
RPL26: 60S ribosomal protein L26
RPS 7: Ribosomal protein S 7
rpP0: Ribosomal phosphoprotein PO
RRM2B: Ribonucleoside-diphosphate reductase subunit M2 B
RYBP: Ring YY1 Binding Protein
SACE: Serum angiotensin-converting enzyme
SERPINF2: serine proteas inhibitor serpin peptidase inhibitor,
SNP309: single nucleotide polymorphism at nucleotide 309
SNPs: single-nucleotide polymorphisms
SOD2: superoxide dismutase 2
SRD5A2: Steroid 5-alpha reductase type II
TBRI: Theodor Bilharz Research Institute
TEK: Angiopoietin-1 receptor
TERT: Telomerase reverse transcriptase
TGFα : transforming growth factor-α
TGF-β_1: Transforming growth factor β_1
THBS1: Thrombospondin 1
TIAM1: T-cell lymphoma invasion and metastasis-inducing protein 1
TIMP: Tissue inhibitors of metalloproteinase
TIMP-1: Tissue inhibitors of metalloproteinase-1
TNF-α: Tumour necrozing factor-α
TOP2A: DNA topoisomerase 2-alpha
TTR: transthyretin
VCAM-1: Serum vascular-cell adhesion molecules
VEGF: Vascular endothelial growth factor
WBCs: White blood cells
XLKD1: Extracellular link domain containing 1

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Background: **The murine double minute 2 (MDM2), an oncogene, acts as a major regulator of the p53 protein. A polymorphism in the MDM2 promoter, SNP309 (T/G), has been shown to alter protein expression and may thus play a role in carcinogenesis. MDM2 is a nuclear phosphoprotein that binds to TP53 and inhibits TP53-dependent transcription.**

Aim of the work: **This study aims to evaluate the association of the MDM2 promoter SNP309 and development of hepatocellular carcinoma among Egyptian patients with chronic viral hepatitis.**

Subjects and Methods: **MDM2 SNP309 (T/G) genotyping was assayed in 67 HCC patients on top of HCV infection and 36 chronic viral hepatitis patients. MDM2 SNP309 genotyping was assayed using realtime polymerase chain reaction (PCR)**

Results: **The frequency of the distribution pattern of the MDM2 SNP309 TT, TG, and GG genotypes in HCC group were 38.8%, 50.7% and 10.4% respectively and that in the control group were 38.9%, 50% and 11.1% respectively. There was with no significant difference between the two studied groups with [p value 0.994]. There were no significant difference in the distribution pattern of the SNP309 (TT+TG versus GG or TG+GG versus TT genotypes between the studied two groups with P value 0.917 and 0.993 respectively. There was no significant difference between the MDM2 SNP309 genotypes and the type of viral infection whether it was HCV or HBV. There was no significant difference between the MDM2 SNP309 genotypes regarding the tumour site, size or number. The male:female ratio in HCC group was 4.2 and that in the control group was 1.4. AFP level was highly significantly increased in HCC group [40(7.6-164ng/dl)] than that of the control group [5(2.7-7.7ng/dl)] with P value of 0.001. In the control group, the distribution of the genotype (TT, GG and TG) and the prescence of cirrhosis were (85.7%, 100% and 88.9%) with no significant difference [P value 0.725].**

Conclusion: **Current data suggest that the association between MDM2-SNP309 genotype and HCC in the cohort study in Egyptian population is not significant.**

Key words: MDM2 SNP309, chronic hepatitis and hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is one of the most common and aggressive human malignancies worldwide **(Pisani et al., 1999)**. It is the fifth most common neoplasm, the major cause of death in patients with liver cirrhosis, and the third most common cause of cancer-related death in the world **(Parkin et al., 2001, Liovet et al., 2003)**.

Hepatocellular carcinoma (HCC) increased in Egypt in the past years, becoming the most common cancer among men. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the known primary risk factors for HCC **(Schiefelbein et al., 2012)**.

HCV transmission is ongoing in Egypt, and incidence rates have been estimated at 2.4 per 1,000 person-years (165,000 new infections annually) **(Mostafa et al., 2010)**.

More than 170 million patients worldwide are chronically infected with hepatitis C virus (HCV). Prevalence rates range from 0.5% in Northern European countries to 28% in some areas of Egypt **(Bartosch and Dubuisson, 2010)**.

Hepatitis B virus (HBV) infection continues to be a global public health issue. The World Health Organization (WHO) reports that approximately 2 billion people worldwide have been infected with HBV and approximately 350 million individuals suffer from HBV-induced chronic liver disease (Chang et al., 2009). Without intervention, 15% to 40% of chronic HBV-infected individuals will develop cirrhosis, end-stage liver disease, hepatocellular carcinoma (HCC), or will require liver transplantation **(Shepard et al., 2006, Sorrell et al., 2009)**. The mass HBV vaccination program has led to marked reduction in HBV infection and also in the incidence of HCC **(Chang et al., 2009)**.

Hepatocellular carcinoma (HCC) is one of the most aggressive cancers worldwide. In Egypt, the disease is usually detected in an advanced stage at which no treatment may be effective including surgery. Early detection of the disease is thus an important goal allowing the patient to be treated before the enlargement of the tumor or its metastasis to distant organs. Tumor markers are serological agents which its serum level may be useful in predicting the presence of the tumor at early stages **(El-Tayeh et al., 2012)**.

However, identification of HCC in high-risk patients, who are at a potentially curable stage, is still an unsolved clinical problem. At present, assessment of AFP levels in conjunction with ultrasound is the "standard procedure" for screening of high-risk patients **(Yagmur et al., 2007)**. As elevated AFP is found only in 40%-75% of patients with HCC **(DiBisceglie et al., 1988, Sherman et al., 1995, Gebo et al., 2002)** and the reported diagnostic value of AFP for detecting HCC by using the most commonly reported cutoff value (AFP level > 20 μg/L) ,its sensitivity is 41% to 65% and the specificity is 80% to 94% **(Gupta et al., 2003)**.

Many studies infer that the variations in HCC development not only depend on somatic mutations occurring in the tumor itself but also on host genetic **factors (Yoon et al., 2008, Ezzikouri et al., 2009, Acun, et al., 2010)**.

Hepatocellular carcinoma exhibits numerous genetic abnormalities (including chromosomal deletions, rearrangements, aneuploidy, gene amplifications, and mutations), as

well as epigenetic alterations (including modulation of DNA methylation). The combination of genetic and epigenetic alterations activates positive mediators of cellular proliferation (including cellular protooncogenes and their mitogenic signalling pathways) and inactivates negative mediators of cellular proliferation (including tumor suppressor genes), resulting in cells with autonomous growth potential. The current knowledge of molecular signatures of early HCC is preliminary and further studies are required to elucidate hepatocarcinogenesis in concordance with histopathological classification of early HCC. Many of the investigations have presented promising progress in the use of gene expression profiling in elucidating the molecular pathogenesis of HCC. Gene expression profiling studies will allow identification of molecular signatures of HCC that are useful as screening and surveillance tools and for predicting the risk of HCC development in cirrhotic tissue and this will eventually lead to personalized treatment for HCC in the broader picture **(Suriawinata and Thung, 2010)**.

The murine double minute 2 (MDM2) is an important regulator of tumor development. MDM2, an E3 ubiquitin ligase, regulates p53 by controlling both the stability of the p53 protein and the activity of p53 that a transcription factor **(Marine et al., 2006)**. The importance of the p53 tumor suppressor pathway for the prevention of transformation has long been recognized, as inactivation of the pathway is a frequent step in the development of the majority of human and murine cancers **(Hollstein et al., 1994, Sherr, 2006)**.

A polymorphism in the MDM2 promoter, single nucleotide polymorphism at nucleotide 309 (SNP309) (T/G), has been shown to alter protein expression and may thus play a role in carcinogenesis **(Acun, et al., 2010)**.

In fact, numerous studies have shown that over expression of MDM2 is an important event in carcinogenesis; in addition, MDM2 amplification occurs mostly in the absence of p53 mutation, supporting the concept that MDM2 amplification and p53 mutation are alternative mechanisms of p53 dysfunction **(Oliner et al., 1993, Reifenberger et al., 1993, Momand et al., 1998)**. Furthermore, SNP309 has been shown to be associated with earlier age of onset of certain hereditary and sporadic cancers in human **(Bond et al., 2004, Bougeard et al., 2006)**.

This study aims to evaluate the association of the MDM2 promoter SNP309 and development of hepatocellular carcinoma among Egyptian patients with chronic viral hepatitis.