Comet assay for detection of DNA damage caused by hepatitis C virus induced cirrhosis and hepatocellular carcinoma

Thesis submitted for partial fulfillment of Master degree in clinical and chemical pathology

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LIST OF ABBREVIATIONS

- **2'-5' OAS:** Oligoadenylate synthetase
- **6−4 PPs:** 6−4 pyrimidine-pyrimidone photoproducts
- **8−OHdG:** 8−hydroxydeoxyguanosine
- **8-oxoG:** 8-oxo-7,8-dihydroguanine
- **ADAR:** Adenosine deaminase-RNA-specific
- **ALT:** Alanine aminotransferase
- **Anti-LKM :** Anti-liver-kidney microsomes
- **AP:** Apurinic/ apyrimidinic sites
- **APC:** Antigen-presenting cells (APC)
- **AST**: Aspartate aminotransferase
- **ATM:** Ataxia telangiectasia mutated kinase
- **BER:** Base excision repair
- **CARDIF:** CARD adaptor inducing IFN-β CARDIF
- **cDC:** conventional DC
- **Chk2:** Checkpoint kinase 2
- **CPD:** Cyclobutane Pyrimidine Dimer
- **CTL:** cytotoxic T lymphocyte
- **DAPI:** 4,6-diamidino-2-phenylindole
- **DC:** Dendritic cells
- **DI H2O:** Di-ionized water
- **DNA:** DeoxyriboNucleic Acid
- **DSB:** Double-Strand Break
- **ds-RNA:** double-stranded RNA
- **EB:** Ethidium bromide
- **EDHS:** Egypt Demographic and Health Survey
- **EIA:** Enzyme immunoassay
- **ELISA:** Enzyme-linked immunosorbent assay
- **FCM:** Flow cytometry
- **FISH:** Fluorescence in situ hybridization

- **FoxP3:** Forkhead box P3
- **FPG:** formamidopyrimidine DNA glycosylase
- **GC-MS:** Gas chromatography-mass spectrometry
- **GGR:** Global-Genome Repair
- **GITR:** Glucocorticoid-induced TNF receptor
- **HBsAg:** Hepatitis B surface antigen
- **HBV:** Hepatitis B virus
- **HCC:** Hepatocellular carcinoma
- **HCV:** Hepatitis C virus
- **HIV:** Human immunodeficiency virus
- **HR:** Homologous Recombination
- **HVR:** Hypervariable regions
- **IFN:** Interferon
- **IFNAR:** IFN-α receptor 1
- **IKKε:** IkappaB kinase epsilon
- **IPS-1:** IFN-β promoter stimulator protein 1
- **IRF3:** IFN regulatory factor 3
- **ISDR:** Interferon-sensitivity–determining region
- **ISG:** IFN stimulated gene
- **ISGF3:** ISG factor 3
- **ISREs :** IFN-stimulated response elements
- **JAK⁄STAT:** Janus kinase⁄ signal transducer and activator of transcription
- **LDLR:** Low-density lipoprotein receptor
- **MAVS:** Mitochondrial antiviral signaling protein
- **MGMT:** Methyl guanine methyl transferase
- **MMR:** Mismatch repair
- **NAFLD:** Nonalcoholic fatty liver disease
- **NER:** Nucleotide excision repair
- **NF-kb:** nuclear factor kappa-light-chain-enhancer of activated B cells
- **NHEJ:** Non-Homologous End-Joining
- **NK:** natural killer

- **NS:** Nonstructural region
- **PCR:** Polymerase Chain Reaction
- **pDC:** plasmacytoid DC
- **PI:** Propidium iodide
- **PKR:** Protein kinase R
- **PP2A:** Protein phosphatase 2A
- **Rb:** Retinoblastoma tumor suppressor protein
- **RIA:** Radio immunoassay
- **RIBA:** Recombinant immunoblot assay
- **RIG-I:** Retinoic acid–inducible gene I
- **RNA :** RiboNucleic Acid
- **SCGE:** Single-cell gel electrophoresis-SCGE
- **SOCS:** Suppressor of cytokine signaling
- **SSB:** Single-Strand Break
- **TBK1:** TANK-binding kinase 1
- **TCR:** Transcription-Coupled Repair
- **Th1:**T helper 1
- **Th2:**T helper 2
- **TLR:** Toll-like receptor
- **TLS:** Translesion synthesis
- **TNF:** Tumor necrosis factor
- **Treg:** T regulatory cells
- **TRIF:** TLR3 recruits the adapter molecule Toll–IL-1 receptor domain–containing adaptor inducing IFN-β
- **TRIF:** Toll–IL-1 receptor domain–containing adaptor inducing IFN-β
- **TUNEL:** Terminal deoxyribonucleotidyltransferase mediated deoxyuridine triphosphate nick end labeling assay
- **TYK2:** Tyrosine kinase
- **UTR:** Untranslated regions
- **VISA:** Virus-induced signaling adapter

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Abstract

Background and aim of work: A significant proportion of cancer is attributable to DNA damage caused by chronic infection and inflammation. Because hepatitis C virus (HCV) cause chronic infection and inflammatory disease, the aim of the present study was to investigate DNA damage in patients with HCV infection compared with controls and whether there is an association between the level of peripheral DNA damage and hepatocellular carcinoma (HCC). We also aimed to correlate the findings of DNA damage with the clinical and laboratory parameters and to investigate if the comet score is a possible marker for HCC detection.

Methods: Sixty nine patients with HCV (14 patients without cirrhosis, 28 patients with cirrhosis, 27 patients with HCC) and 29 healthy subjects were included in the study. The DNA damage in lymphocytes was determined using the alkaline comet assay.

Results: Comet scores were significantly higher in HCC and cirrhotic patients compared to healthy controls and HCV patients without cirrhosis. Patients who presented with DNA damage had 4.7 fold risk of having HCC more than those with undamaged DNA. At a cutoff of 215, comet scores had 89% sensitivity for detection of HCC.

Conclusions: Ongoing DNA damage in peripheral blood lymphocytes was detected in complicated HCV patients. Thus such a test could be used to detect which patient is at higher risk to develop HCC.

Key words: Comet assay, Hepatitis C virus, Hepatocellular carcinoma.

INTRODUCTION AND AIM OF WORK

Approximately 1.8 million cases of cancer are estimated to be attributable to infectious agents. Viruses such as the hepatitis B and C viruses (HBV and HCV, respectively) are among the most common agents causing cancer **(Ohshima et al., 2003).** The mechanisms of carcinogenesis associated with infection and inflammation have not been fully elucidated. Three main mechanisms have been proposed to account for infection-associated carcinogenesis: a direct action of the infectious agent on host cells or tissues; immunosuppression; and the production of reactive oxygen and nitrogen species (ROS and RNS, respectively). Prolonged activation of inflammatory cells generate ROS and RNS and these cause tissue and DNA damage that may contribute to the development of cancer **(Ohshima et al., 2003).**

Chronic liver injury leading to necrosis, inflammation and liver regeneration may progress to cirrhosis and hepatocellular carcinoma (HCC). Hepatitis C virus-related liver damage is characterized by an increase in free radical formation, manifested by increased hepatic and serum levels of products of lipid peroxidation, and accumulation of oxidative DNA damage in the liver **(Farinati et al., 1999).**

Hepatitis C virus increases the risk of hepatocellular carcinoma (HCC), thus suggesting the possible existence of a genotoxic mechanism. Evidence supporting this hypothesis, however, is still limited and to some extent controversial. Considering that the HCV virus is known to infect peripheral lymphocytes, a direct genotoxic effect of HCV may suggest that the same genotoxic effect may operate in the liver and contribute to hepatocarcinogenesis.

The comet assay (single-cell gel electrophoresis) is a well-established genotoxicity test. Because of its demonstrated ability to detect various types of DNA damage with high sensitivity in eukaryotic cells exposed to genotoxic agents, this technique is being used with greater frequency in humans **(Kassie et al., 2000)**.

Aim of work

The aim of the study is to investigate peripheral DNA damage by the alkaline comet assay in patients with HCV infection (uncomplicated, complicated with liver cirrhosis and HCC on top of cirrhosis) compared to healthy subjects. We also aim to correlate the findings of DNA damage with the clinical and laboratory parameters and to investigate if the comet score is a possible marker for HCC detection.

CHAPTER 1

HEPATITIS C VIRUS INFECTION

Hepatitis C virus (HCV) infects an estimated 170 million persons worldwide and thus represents a viral pandemic, one that is five times as widespread as infection with the human immunodeficiency virus type 1 (HIV-1) **(Lauer and Walker,2001).**

Structure of HCV virions

HCV circulates in various forms in the serum of an infected host, including (i) virions bound to very–low density lipoproteins and low-density lipoproteins, which appear to represent the infectious fraction; (ii) virions bound to immunoglobulins; and (iii) free virions **(Bradley et al.,1991; Thomssen et al.,1993).** HCV particles are believed to have a diameter of 55 to 65 nm **(Kaito et al.,1994; Shimizu et al.,1996).** By analogy with the known 3D structures of closely related flaviviruses and alphaviruses, HCV is thought to adopt a classical icosahedral scaffold in which its two envelope glycoproteins, E1 and E2, are anchored to the host cell-derived double-layer lipid envelope. Underneath the membrane is the nucleocapsid that likely is composed of multiple copies of the core protein, forming an internal icosahedral viral coat that encapsidates the genomic RNA, *(fig.1),* **(Penin et al.,2004).**