

# بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ





# شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



# جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

## قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها  
علي هذه الأقراص المدمجة قد أعدت دون أية تغييرات



## يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار





# بعض الوثائق الأصلية تالفة





# بالرسالة صفحات لم ترد بالأصل



*Epidermal growth factor (EGF) gene polymorphism as a risk factor for development of hepatocellular carcinoma (HCC) in chronic liver disease patients*

**By**

*Huda Ramadan Reyad Abass*  
*M.B.B.Ch. in medicine and surgery*

**Thesis**

*Submitted for partial fulfillment of MSC degree in Medical Biochemistry*

**Supervised by**

*Prof. Dr. Emad Zaki Abass*  
*Professor of Medical Biochemistry*  
*Faculty of Medicine*  
*Cairo University*

*Prof. Dr. Gamal El-Dein Esmat Mohamed*  
*Professor of Tropical Medicine*  
*Faculty of Medicine*  
*Cairo University*

*Dr. Ghada Mahmoud Abdel-Aziz*  
*Lecturer of Medical Biochemistry*  
*Faculty of Medicine*  
*Beni-Sweif University*

*Beni-Sweif University*  
*Faculty of Medicine*  
**2011**

## *Acknowledgment*

*First and foremost thanks to "Allah" the most beneficial and the most merciful*

*It is a great pleasure to express my profound gratitude and deep thanks to Prof. Dr. Emad Zaki Abass, professor of Medical Biochemistry, faculty of Medicine, Cairo University for his supervision, generous cooperation and great help to end this work,*

*I wish to express my sincere thanks to Prof. Dr. Gamal Esmat, Professor of Tropical Medicine, faculty of Medicine, Cairo University for his careful supervision on this work, his valuable cooperation and encouragement.*

*My deep thanks and grateful acknowledgement to Prof. Dr. Olfat Shaker, professor of Medical Biochemistry, faculty of Medicine, Cairo University for her careful supervision on this work and her help in the practical part of thesis and analysis of results.*

*I am thankful to Dr. Ghada Mahmoud Abd El-Aziz, lecturer of Medical Biochemistry, faculty of Medicine, Beni-Sweif University, for her support and help during this work,*

*I would like to express my deep thanks to all staff members in Medical Biochemistry department for their help and support.*

*I want to thank the patients and healthy volunteers for their cooperation.*

*Finally, my deepest thanks to my family for all their help and support.*

## ***Abstract***

**Background:** Overexpression of epidermal growth factor (EGF) in the liver induces transformation to hepatocellular carcinoma(HCC) in animal models. Polymorphisms in the EGF gene modulate EGF levels.

**Objective:** to evaluate the effect of EGF gene single nucleotide polymorphism and assess its correlation with risk of hepatocellular carcinoma in patient with chronic liver diseases.

**Patients and Methods:** The present study included 100 subject, divided into 4 groups; Group 1: included 17 asymptomatic healthy control volunteers who matched with age and sex, Group 2: included 20 patients with chronic hepatitis C infection, Group 3: included 20 patients with liver cirrhosis and Group 4: included 20 patients with hepatocellular carcinoma. For all subjects the following investigations were performed; complete blood count, Liver functions tests. Sero markers of hepatitis viruses HBsAg, total anti-HBC, and HCV-RNA by quantitative PCR and Alfa fetoprotein, in addition to full history taking including risk factors of HCV infection, thorough clinical examination and abdominal ultrasonographic examinations were done. One ml blood was taken on EDTA for DNA extraction and detection of single nucleotide polymorphism of the EGF by polymerase chain reaction (PCR) and restriction enzymes followed by agarose gel electrophoresis stained with ethidium bromide.

**Results:** The control subjects with GG and GA genotypes had 7.875 and 3.375 folds odds ratio for developing HCC and the control group with GG and GA genotypes had 2.571 and 1.929 folds for developing cirrhosis than AA genotype respectively. The cirrhotic patients with GG and GA genotypes had 3 and 1.75 folds for developing HCC respectively and the chronic hepatitis C patients with GG genotype had 2.333 folds of developing HCC than AA genotype.

**Conclusion:** The EGF 61 A/G polymorphism might be associated with a high risk for the development of HCC in both chronic liver disease patients and normal healthy subjects and a risk factor for cirrhosis development in normal healthy subjects.

**Keywords:** Epidermal growth factor, Chronic liver disease, Polymorphism and Hepatocellular carcinoma

## List of Contents

|  | Page |
|--|------|
| List of Tables .....   | I    |
| List of Figures .....  | II   |
| List of Abbreviations .....  | III  |
| Introduction & Aim of the work .....                                     | ١    |
| Review of literature   |      |
| Chapter (1): Hepatocellular Carcinoma (HCC) .....                        | ٤    |
| Chapter (2): Genetics of HCC .....                                       | ٢١   |
| Chapter (3): Epidermal Growth Factor (EGF) .....                         | ٣١   |
| Chapter (4): EGF gene single nucleotide polymorphism (SNP) and HCC ..... | ٤٩   |
| Chapter (5): Targeted Therapies in HCC .....                             | ٧٤   |
| Subjects & Methods .....   | ٧٩   |
| Results .....  | ٨٧   |
| Discussion & Recommendations .....                                       | ١٠٢  |
| Summary & Conclusion .....   | ١١٣  |
| References .....   | ١١٧  |
| Arabic summary   |      |

## List of Tables

| <b>Table</b> |   | <b>Page</b>  |
|--------------|---|--------------|
| <b>(1)</b>   | Annual incidence of hepatocellular carcinoma per 100,000 populations  | <b>(6)</b>   |
| <b>(2)</b>   | Staging systems in hepatocellular carcinoma   | <b>(14)</b>  |
| <b>(3)</b>   | The clinical data of all studied groups (Mean and SD)   | <b>(89)</b>  |
| <b>(4)</b>   | Genotypes, carriers and alleles in all groups   | <b>(95)</b>  |
| <b>(5)</b>   | Relation of epidermal growth factor genotypes to different parameters studied in all groups                                     | <b>(98)</b>  |
| <b>(6)</b>   | Risk estimation in HCC, cirrhosis and hepatitis c groups in relation to control group as regard genotypes, carriers and alleles | <b>(100)</b> |
| <b>(7)</b>   | HCC risk estimation in both cirrhotic and hepatitis c groups as regard genotypes, carriers and alleles                          | <b>(102)</b> |

## List of figures

| Figure |   | page     |
|--------|---|----------|
| (1)    | Chromosomal localization of epidermal growth factor gene.                                 | (31)     |
| (2)    | Schematic representation of human kidney EGF precursor cDNA and protein.                  | (34)     |
| (3)    | Exon organization and protein domains in the human EGF precursor gene.                    | (35)     |
| (4)    | The synthetic $\beta$ - ueogasterone gene showing the nucleotide and amino acid sequence. | (37)     |
| (5)    | Rainbow structure of the mouse epidermal growth factor.                                   | (37)     |
| (6)    | EGF structure and position of disulfide bond.   | (39)     |
| (7)    | Key components of the EGF pathway.  | ١٤)<br>( |
| (8)    | Overview of the EGF signaling pathway.  | (44)     |
| (9)    | A/G Single nucleotide polymorphism.   | (50)     |
| (10)   | Agarose gel electrophoresis.  | ٨٦)<br>( |
| (11)   | Percentage distribution of liver size in all studied groups.                              | (٩١)     |
| (12)   | Percentage distribution of spleen size in all studied groups.                             | ٩٢)<br>( |
| (13)   | Percentage distribution of HBsAg in all studied groups.                                   | (٩٣)     |
| (14)   | Percentage distribution of HCV antibodies in all studied groups.                          | ٩٤)      |
| (15)   | Percentage distribution of all groups as regarding genotypes distribution.                | ٩٦)      |
| (16)   | Percentage distribution of all groups as regarding G and A alleles.                       | ٩٦)      |

|      |  |      |
|------|--|------|
| (17) | Percentage distribution of all groups as regarding (GG GA carriers). | (۹۷) |
| (18) | Age distribution in different genotypes.                             | ۹۹)  |
| (19) | Sex distribution in different genotypes.                             | ۹۹)  |

## List of abbreviations

|                        |   |
|------------------------|---|
| <b>A</b>               | Adenine   |
| <b>A:T</b>             | Adenine:Thymine   |
| <b>AAF</b>             | Acetylaminofluorene   |
| <b>ADAM</b>            | A disintegrin and metalloprotease   |
| <b>AFB1</b>            | Aflatoxin B1  |
| <b>AFP</b>             | Alpha Fetoprotein   |
| <b>AKT</b>             | Ak, name for a mouse strain developing spontaneous thymic lymphomas t" stands for 'transforming' (anti-apoptotic serine-threonine kinase) |
| <b>ANGII</b>           | Angiotensin 2   |
| <b>ALT</b>             | Alanine aminotransferase  |
| <b>ALP</b>             | Alkaline phosphatase  |
| <b>AR</b>              | Amphiregulin  |
| <b>AST</b>             | Aspartate aminotransferase  |
| <b>ATP</b>             | Adenosine triphosphate  |
| <b>BCL2</b>            | B-cell lymphoma 2   |
| <b>BCLC</b>            | Barcelona-clinic liver cancer   |
| <b>BMI</b>             | Body mass index   |
| <b>BP</b>              | Base pair   |
| <b>BTC</b>             | Beta-cellulin   |
| <b>C</b>               | Cytosine  |
| <b>CCL4</b>            | Carbon tetrachloride  |
| <b>CDK</b>             | Cyclin dependent kinase   |
| <b>cDNA</b>            | Complementary deoxyribonucleic acid   |
| <b>C-FOS</b>           | Cellular Finkel–Biskis–Jinkins murine osteogenic sarcoma virus  |
| <b>Chemokine MIP-3</b> | Chemokine macrophage-inflammatory protein-3   |
| <b>CLIP</b>            | Cancer of the Liver Italian Program   |
| <b>C-MYC</b>           | Cellular myelocytomatosis oncogene  |

|                     |  |
|---------------------|--|
| <b>COS 7</b>        | cercopithecus aethiops (African green Monkey)<br>fibroblast- like kidney cells 7 |
| <b>COX-2</b>        | Cyclooxygenase 2   |
| <b>C-P A</b>        | Child-pugh a   |
| <b>CREB</b>         | CAMP response element-binding  |
| <b>c-SRC (sarc)</b> | Cellular- Short for sarcoma  |
| <b>CTGF</b>         | Connective tissue growth factor  |
| <b>CUPI</b>         | Chinese university prognostic index  |
| <b>DNA</b>          | Deoxyribonucleic acid  |
| <b>ECM</b>          | Extra cellular matrix  |
| <b>EDTA</b>         | Ethelene diamine tetra acetic acid   |
| <b>EGF</b>          | Epidermal growth factor  |
| <b>EGFR</b>         | Epidermal growth factor receptor   |
| <b>EIF 2B</b>       | Eukaryotic initiation factor 2 B   |
| <b>EPG</b>          | Epigen   |
| <b>ER</b>           | Estrogen receptor  |
| <b>ERBB</b>         | Erythroblastosis binding   |
| <b>EREG</b>         | Epiregulin   |
| <b>ERK</b>          | Extracellular signal regulated kinase  |
| <b>FAS</b>          | Factor of apoptosis  |
| <b>FASL</b>         | Fas ligand   |
| <b>FDA</b>          | Federal drug agency  |
| <b>G</b>            | Guanine  |
| <b>G : C</b>        | Guanine: cytosine  |
| <b>GRB2</b>         | Growth factor receptor bound protein   |
| <b>GAP</b>          | Gtpase activation protein  |
| <b>GDP</b>          | Guanosine diphosphate  |
| <b>GPCR</b>         | G protein coupled receptor   |
| <b>GTP</b>          | Guanosine triphosphate   |
| <b>H- RAS</b>       | Harvey ras   |
| <b>HB-EGF</b>       | Heparin-binding EGF  |
| <b>HBsAG</b>        | Hepatitis B surface antigen  |

|                               |  |
|-------------------------------|--|
| <b>HBV</b>                    | Hepatitis B virus  |
| <b>HBX</b>                    | Hepatitis B virus X protein                                |
| <b>HCC</b>                    | Hepatocellular carcinoma                                   |
| <b>HCV</b>                    | Hepatitis C virus  |
| <b>HCV ab</b>                 | HCV antibodies   |
| <b>HER</b>                    | Human epidermal growth factor receptor                     |
| <b>HSCS</b>                   | Hematopoietic stem cells                                   |
| <b>IAPS</b>                   | Inhibitor of apoptosis proteins                            |
| <b>IG EGF</b>                 | Immunoglobulin epidermal growth factor                     |
| <b>IGF</b>                    | Insulin-like growth factor                                 |
| <b>IGF-1R</b>                 | IGF-1 receptor   |
| <b>IGG</b>                    | Immunoglobulin G   |
| <b>IGM</b>                    | Immunoglobulin M   |
| <b>IL-8</b>                   | Intrleukin 8   |
| <b>IL-1<math>\beta</math></b> | Interleukin-1beta  |
| <b>IL-6</b>                   | Interleukin-6  |
| <b>IL-1</b>                   | Interleukin-1  |
| <b>INF<math>\gamma</math></b> | Interferon-gamma   |
| <b>JAK</b>                    | Janus kinase   |
| <b>JIS</b>                    | Japan integrated staging                                   |
| <b>JNK</b>                    | C-Jun N-terminal protein kinase                            |
| <b>KBP</b>                    | Kilo base pair   |
| <b>KCS</b>                    | Kupffer cells  |
| <b>KDA</b>                    | Kg dalton  |
| <b>K-RAS</b>                  | Kirsten RAS  |
| <b>LOH</b>                    | Loss of heterozygosity                                     |
| <b>LPS</b>                    | Lipopolysaccharide   |
| <b>M.B.B.CH</b>               | Medicinae Baccalaureus, Baccalaureus Chirurgiae            |
| <b>M TOR</b>                  | Mammalian Target of Rapamycin                              |
| <b>MAPK</b>                   | Mitogen activated protein kinase                           |
| <b>MEK</b>                    | MAP/Erk kinases  |
| <b>MET Codon</b>              | Methionine codon   |
| <b>MKKS</b>                   | MAP kinase kinases   |
| <b>MNK</b>                    | Mapk (mitogen-activated protein kinase)-interacting kinase |