Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Anbar / College of Science
Department of Chemistry

Molecular and Biochemical Study in Chronic Hepatitis B Patients in Anbar Province.

A Dissertation

Submitted to the Council of the College of Science, University of Anbar in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemistry Science

By

Ban Mohammed Abbas AL-Temimi

B.Sc. Chemistry, College of Science, University of Anbar (2000)
M.Sc. Chemistry, College of Science, University of Anbar (2013)

Supervised by

Professor	Assistant Professor
Dr. Mushtak.T. S. Al-Ouqaili	Dr. Khald. F. A. AL-Riwe

2020 A.D 1441 A.H

بسم الله الرحمن الرحيم

قُل لَّوْ كَانَ الْبَحْرُ مِدَادًا لِّكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَن تَنفَدَ كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا (901)

صدق الله العظيم

سورة الكهف / الآية (901)

Supervisors Certification

*We certify that the preparation of this thesis entitled " **Molecular and Biochemical Study in Chronic Hepatitis B Patients in Anbar Province** " is prepared under our supervision at Department of Chemistry College of Science University of Anbar as a partial requirement for the **Degree of Ph.D. in Chemistry.***

Signature:

(Supervisor)
Prof. **Dr. Mushtak.T.Al-Ouqaili**
College of Medicine
University of Anbar
Date: / /2020

Signature:

(Co-advisor)
Assist. Prof. **Dr. Khalid. F. AL-Rawi**
College of Science
University of Anbar
Date: / /2020

Chairman of the Postgraduate Studies Recommendation:

In view of the available recommendations, I forward this thesis for debate by the examining committee.

Signature:

Assist prof. Dr. Ahmed Mishaal Mohammed
Chairman of the Postgraduate Studies Committee, Department of Chemistry. University of Anbar/ College of Science.
Date: / / 2020

Committee Certification

We, the members of the examining Committee, Certify that after reading this thesis ***"Molecular and Biochemical Study in Chronic Hepatitis B Patients in Anbar Province"*** *and have examined the student* ***(Ban Mohammed Abbas)*** *in its contents, that in our opinion, it is accepted as a thesis for the* ***degree of doctor of philosophy in chemistry***

Chairman
Signature:
Name: **Dr. Hussein Kadhem AL-Hakeim**
Scientific degree: Prof. Dr
Date: / / 2020

Member
Signature:
Name: **Dr. Wajeeh Younus Mohammed**
Scientific degree: Prof. Dr
Date: / / 2020

Member
Signature:
Name: **Dr. Hula Younis Fadhil**
Scientific degree: Assist. Prof
Date: / / 2020

Member
Signature:
Name: **Dr. Rashied Mohammed Rashied**
Scientific degree: Assist. Prof
Date: / / 2020

Member
Signature:
Name: **Dr. Hameed Hussein Ali**
Scientific degree: Assist. Prof
Date: : / / 2020

Member (Supervisor)
Signature:
Name: **Dr. Mushtak Talb Salih**
Scientific degree: Prof. Dr
Date: / / 2020

Member (Supervisor)
Signature:
Name: **Dr. Khalid Farouq Abdul-Khafur**
Scientific degree: Assist. Prof
Date: / / 2020

Approval of Dean

Signature:
Name: Prof. Dr. **Emad A. Mohammed Salih**
Dean of the College of Science
Date: / / 2020

Acknowledgements

First of all, I would like to thank our God for His mercy and continuous help in my life.

I would like to express my deepest appreciation and sincere gratitude to my supervisor, Professor Dr. Mushtak T.S. Al-Ouqaili for his supervision, scientific guidance, generous help, support and precious advice through the research period which contributed in carrying out this scientific work.

Most profound thanks and gratitude are presented to my supervisor, Assist Professor Dr. Khalid. F. AL-Rawi for his supervision and for his guidance, fruitful scientific instructions and constant cooperation. Iam really grateful for their encouragement, and effort throughout the work.

Special thank and gratitude are due to the Deanery of the College of Science, University of Anbar, and the Head of Department of Chemistry Professor Dr. Ahmed Mishaal Mohammed, the teaching staff members and all supportive staff in the Department.

I express my grateful thanks to my friend Dr. Sahar, General AL-Ameria Hospital/AL-Anbar Province for his assistance in providing the researchers with clinical specimens and all staff of Al-Ameria hospital. Also, special thanks are due to Prof. Dr. Fawzi Kadhem for carrying out the statistical analysis of this work. Thanks are also extended to all staff of Dar-Al-Shefaa Private Laboratory, and AL-Tamyaz private Laboratory.

Finally, I wish to express my deep gratitude for my husband Dr. Jumaah and my family without their support for me I can't complete my study.

Ban

Dedication

*To **My Parents** who taught me that ambition has no limits, and who that supported and stood beside me all the time.*

*To the tender hearts which surround me with care and courage, my **sisters** and **brothers**.*

To whom by his side, I have found all that I have been waiting for, and I can't ask anything more

***My husband** (Jumaah Ayfan).*

And to all those who suffer to raise the name of Iraq, and

*To my country.... **IRAQ***

I dedicate my efforts

Summary

Hepatitis B is a potentially life-threatening liver infection caused by a specific type of virus called hepatitis B virus (HBV). Iraq is among the intermediate HBV endemic countries because the infected rate of HBV is between (3%-4.5%) among the population and (2%-3%) among apparently healthy blood donors; therefore, this study was designed. A total of 40 patients with infectious phases of hepatitis B (HBeAg positive) divided into 20 female and 20 male and 40 non-infectious phases of hepatitis B patients (HBeAg negative) 18 female and 22 male and 40 healthy subjects 19 female and 21 male were included in this study during the period from February to September 2018. The study was carried out in the General Al-Ameria Hospital/ Al-Anbar province.

Hepatitis B virus was identified as preliminary by Rapid immune chromatographic assay. Fully automated chemistry analyzer TOSOH, AIA-1800 ST, for the detection of HBsAg level was also done as a confirmatory test. Further, assay procedure for biochemical tests including Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Also total serum Bilirubin (TSB), Gamma-glutamyl transferase (GGT) and Total protein Albumin and Globulin were done by using automatic chemistry analyzer instrument (MNCHIP) for each serum specimen from patients and healthy individuals. On the other hand, measurement of non-enzymatic antioxidant which includes Vitamin C (V.C), and enzymatic antioxidant which includes superoxide dismutase (SOD). Further, the production of lipid peroxidation marker malondialdehyde (MDA) in addition interlukin-1 alpha (IL-1α), and

interlukin-2 (IL-2) were also achieved by Enzyme- linked immunosorbent assay technique.

Hepatitis B-DNA extraction was done by SaMag-12 automatic nucleic acid extraction system. Quantitative HBV-DNA was amplified using Real-Time PCR. A total of 80 patients were revealed to be positive for HBsAg . Serum level of HBsAg was significantly increased in HBeAg positive patients compared to HBeAg negative patients (7779.9 ± 3898 vs. 3233.8 ± 2474 IU/ml) respectively. The viral load of HBV-DNA was also considerably increased in HBeAg positive patients compared to HBeAg negative patients (35328825 ±23101537 vs. 3115.1±1916.8 IU/ml).

The chromatographic immunoassay was performed for all HBsAg positive patients to investigate the following markers: HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb. In infectious form of hepatitis B, (those patients who were positive for HBeAg), Anti-HBsAg was not detected in any patients. Also, all patients (100%) were anti-HBs negative, in addition to that Anti-HBeAg was detected in 2.5% of patients while 97.5% of them were anti-HBeAg negative. Further, anti-HBc was detected in 67.5% of study patients.

In biochemical and physiological aspect, the statistical analysis revealed that there was a significant increase in the mean level of serum AST, ALT, ALP in (HBeAg-positive) patients compared to (HBeAg negative) patients group and healthy control (P = 0.001). Regarding GGT levels increase in (HBeAg positive), and (HBeAg negative) patients group compared to healthy control (P value=0.002). Also, the level of total serum bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, and

globulin showed no significant difference results in each HBeAg positive and HBeAg negative patients and healthy control.

In oxidative stress markers; vitamin C levels in HBeAg-positive patients were lower than those observed in HBeAg-negative patients or in healthy volunteers (P value=0.001). However, SOD and MDA levels were significantly lower in both HBeAg-positive and HBeAg-negative patients than in the healthy volunteers.

In immunological part, IL-1α levels appeared similar in the three groups of individuals. IL-2 levels were not significantly different between HBeAg-positive and control individuals but HBeAg-negative individuals demonstrated significantly higher levels than either HBeAg-positive patients or the controls (both, $P = 0.001$).

The study concluded that qRT-PCR test have the superior clinical advantage among other techniques in quantitation viral load through study groups. Also, the biochemical parameters, especially ALT, AST, GGT, and ALP, were elevated in HBV patients significantly. The activities of vitamin C were significantly decreased in HBeAg positive patients while the activities of superoxide dismutase was significantly reduced in each HBeAg positive and HBeAg negative patients compared to healthy control. The activity of malondialdehyde has been markedly reduced in HBV patients. Further, Serum levels of IL-2 were decreased in HBeAg positive patients group relative to HBeAg negative patients.

LIST OF CONTENTS

Title	Page
Chapter One/Introduction and Literature Review	

Title	Page
1. 1 Introduction ………………………………………………	1
1.2. Literature Review …………………………………….…	4
1.2.1 The liver ………………………….…………….	4
1.2.2 Liver diseases …………........................	5
1.2.2.1 Hepatitis ……………………….……………....	5
1.2.2.1.1 Viral Hepatitis ………………………………………………	5
1.2.2.1.1.1 Hepatitis B virus (HBV) ………………………………....	6
1.2.2.1.1.2 Morphology of HBV ……………………………..….	7
1.2.2.1.1.3 Clinical types of HBV infection …………………….………	8
1.2.2.1.1.4 Laboratory diagnosis of hepatitis…….................	10
1.2.2.1.1.5 Markers of hepatitis B and its significance to diagnosis criteria ……………………….…………….	11
1.2.2.1.1.6 Hepatitis B DNA (HBV DNA) ………………..….	13
1.2.2.1.1.6.1 Quantitation real time PCR ……......................	13
1.3 Liver function tests as biochemical markers …………………	14
1.3.1 Tests for the liver's ability to transport …………………….	15
1.3.1.1 Serum bilirubin ………………..……..….	15
1.3.2 Tests to detect damage to hepatocytes ….………….	16
1.3.2.1 Alanine aminotransferase and aspartate aminotransfera (ALT and AST) ………………………………………………….	16
1.3.2.2 Alkalin phosphatase (ALP) ………..………………	18

1.3.2.3 γ- glutamyl transferase (GGT)………………………. 18
1.3.3 Tests of the biosynthetic capacity of the liver………. 19
1.3.3.1 Total protein ……………………………………………. 20
1.3.3.1.1 Albumin…………………………………………………. 20
1.3.3.1.2 Globulins ……………………………………………… 21
1.3.3.2 Prothrombin Time (PT)…………………………….. 21
1.3.3.3 Fibrinogen ……………………………………………. 22
1.4 Oxidative stress …………………………………………… 23
1.5 Free radicals ……………………………………………… 24
1.5.1 The source of ROC ……………………………………. 25
1.5.1.1 Endogenous source of ROC …………………………. 25
1.5.1.2 Exogenous source of ROC ………………………… 26
1.6 Lipid peroxidation ………………………………………. 27
1.6.1 Malondialdehyde (MDA)…………………………… 28
1.7 Antioxidants …………………………………………………. 29
1.7.1 Enzymatic antioxidant ……………………………….. 29
1.7.1.1 Superoxide dismutase (SOD) ……………………………….. 29
1.7.2 Non-enzymatic antioxidant 30
1.7.2.1 Vitamin C ………………………………………………….. 31
1.8 Immune system ………………………………………………. 33
1.9 Inflammation ………………………………………………. 34
1.10 Cytokines ………………………………………………….. 34
1.10.1 Types of cytokines ………………………………………. 35
1.10.1.1 Interleukin 1 (IL-1) ………………………………… 36
1.10.1.2 Interleukin 2 (IL-2) ………………………………… 37

1.11 Role of cytokines in viral hepatitis ……………………… 38
1.12 Treatment and vaccination of hepatitis B virus ………. 39
1.13 Aims of study ……………………………………………………….. 41

Chapter Two/ MATERIALS AND METHODS

2.1. Materials ……………..……………………………..……… 42
2.1.1 Chemicals and their sources …………………….…..…… 42
2.2. Subjects ……………………………………………….…………………. 43
2.3 Specimens collection.…………………………..…... 44
2.4 Methods ……………………………………………………….…………….. 44
2.4.1 Serological investigation …………………….………………. 44
2.4.1.1 Preliminary screening of HBsAg patients …………... 44
2.4.1.2 Hepatitis B profile...……………………………. 45
2.4.2 Molecular part of the study ………………………….………..… 47
2.4.2.1 Automated Nucleic acid (HBV-DNA) Extraction …………… 47
2.4.2.2 DNA concentration and purity …………………… 48
2.4.2.3 qRT-PCR Quantification of HBV-DNA using smart cycler II. 48
2.4.2.3.1 Reagent preparation for amplification …………… 49
2.4.2.3.2 Programming of smart cycler II instrument……. 50
2.4.2.3.3 Results Analysis …………………………. 51
2.4.2.3.4 Results Interpretation ……………………………. 51
2.4.3 Biochemical Investigation (liver function test)……… 52
2.4.4 Oxidative stress markers ……………….……… 53
2.4.4.1 Assay Procedure for Human Vitamin C (VC)………. 53
2.4.4.2 Assay Procedure for Human super oxide dismutase (SOD). 55

2.4.4.3 Assay Procedure for Human Malondialdehyde (MDA) 57
2.4.5 Cytokines parameters ………………………………………….. 59
2.4.5.1 Assay Procedure for Human Interleukin-1 (IL-1α) ……… 59
2.4.5.2 Assay Procedure for Human Interleukin-2 (IL-2)……. 63
2.4.6 Hematological Tests ………………………………….. 68
2.4.6.1 Complete Blood Count Analyzer (CBC)…………………… 68
2.4.6.1.1 Sample …………………………………………………………… 68
2.4.6.2 Determination of Prothrombin Time (PT)……………… 68
2.4.6.3 Determination of Fibrinogen ………………………… 69
2.5 Statistical Analysis ………………………………………………. 70

Chapter Three/RESULTS AND DISCUSSION

3.1 Demographic presentation of study sample ……………. 71
3.1.1 Gender ……………………………………………………….. 71
3.1.2 Age ………………………………………………………………… 71
3.2 Virological Markers ……………………………………….. 74
3.2.1 Total patients ………………………………………………. 74
3.2.2 Patients Distribution by Gender ……………………………. 80
3.2.3 Patients Distribution by Age groups ……………………….. 81
3.3 Biochemical Investigation (liver function test) ………………. 83
3.3.1 Total patients ……………………………………………………. 83
3.3.2 Patients Distribution by Gender ……………………………… 87
3.3.3 Patients Distribution by Age groups ………………………. 88
3.4 Oxidative stress parameters ………………………………… 91
3.4.1 Total patients …………………………………………………… 91
3.4.2 Patients Distribution by Gender …………………………. 94

3.4.3 Patients Distribution by Age groups 95
3.5 Cytokines Parameters 98
3.5.1 Total patients 98
3.5.2 Patients Distribution by Gender 100
3.5.3 Patients Distribution by Age groups 101
3.6 Hematological Tests 103
3.6.1 Total patients 103
3.6.2 Patients Distribution by Gender 108
3.6.3 Patients Distribution by Age groups 110
3.7 Correlation of Biomarkers 114
3.7.1 Correlation among biomarkers in HBV (HBeAg positive) and HBV (HBeAg negative) patients 114

CONCLUSIONS & RECOMMENDATIONS

Conclusions 118
Recommendations ….. 120

REFERENCES

References 122

APPENDIX

Appendix.. 158

ARABIC SUMMARY

Arabic Summary... 1

LIST OF TABLES

No.	Title	Page
1.1	Reactive oxygen species and Reactive nitrogen species in the human body	24
2.1	Chemicals and their sources.	42
2.2	Instruments and their manufacture company	43
2.3	Program of RT-PCR for HBV using smart cycler Instrument	51
2.4	Concentration of the Quantitative standards HBV and IC	51
3.1	Distribution of patients and controls according to gender	71
3.2	Distribution of patients and controls according to age	72
3.3	Incidence of hepatitis B serological markers in patients positive and negative results for hepatitis B envelop antigens and in healthy control.	74
3.4	Hepatitis B virus surface antigen (HBsAg) levels and viral load (HBV-DNA copies) in hepatitis B virus envelope antigen-positive and -negative patients.	75
3.5	Hepatitis B virus surface antigen (HBsAg) levels and viral load (HBV-DNA copies) in hepatitis B virus envelope antigen-positive and negative patients distributed by gender.	80
3.6	Hepatitis B virus surface antigen (HBsAg) levels and viral load (HBV-DNA copies) in hepatitis B virus envelope antigen-positive and -negative patients distributed by age groups.	82
3.7	Serum biochemical investigations in both of two study groups (HBeAg positive and negative) Patients and healthy subjects	84
3.8	Serum biochemical investigations in both of two study groups (HBeAg positive and negative) Patients distributed by gender.	87
3.9	Serum biochemical investigations in both of two study groups (HBeAg positive and negative) Patients distributed by age groups.	90
3.10	Serum levels of vitamin C, superoxide dismutase, and malondialdehyde healthy individuals and in patients with and without hepatitis B envelop antigen.	91
3.11	Serum levels of vitamin C, superoxide dismutase, and malondialdehyde in patients with and without hepatitis B envelop antigen distributed by gender.	94

3.12	Serum levels of vitamin C, superoxide dismutase, and malondialdehyde in patients with and without hepatitis B envelop antigen distributed by age groups.	96
3.13	Interleukin -1α and interleukin -2 levels in patients positive and negative for hepatitis B envelope antigens and in healthy individuals.	98
3.14	Interleukin -1α and interleukin -2 levels in patients positive and negative for hepatitis B envelope antigens distributed by gender	100
3.15	Interleukin -1α and Interleukin -2 levels in patients positive and negative for hepatitis B envelope antigens distributed by age groups.	102
3.16	Hematological investigations in both of two study groups (HBeAg positive and negative) Patients and healthy subjects	105
3.17	Hematological investigations in hepatitis B patients according to gender.	109
3.18	Hematological investigations in hepatitis B patients according to age groups.	112
3.19	Correlation of study biomarkers in HBV (HBeAg positive) patients	116
3.20	Correlation of study biomarkers in HBV (HBeAg negative) patients	117

LIST OF FIGURES and SCHEMES

No.	Title	Pages
1.1	Schematic diagram of hepatitis B virion	8
1.2	Schematic diagram of ɣ- Glutamyl cycle	19
1.3	Chemical structure of Malondialdehyde	29
1.4	Generation of reactive oxygen species	30
1.5	Chemical structure of Vitamin C	32
3.1	The amplification curve of HBV-DNA genome in RED Fluorescent channel detected by Real Time polymerase chain reaction	78
3.2	The amplification curve of HBV-DNA genome in FAM Fluorescent channel detected by Real Time polymerase chain reaction	79

List of Abbreviations

Abbreviation	Description
AHB	Acute hepatitis B
ANOVA	Analysis of variance
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ALP	Alkaline phosphatase
ATP	Adenosine triphosphate
CBC	Complete blood count
CHB	chronic hepatitis B
CD4+	cluster of differentiation 4
CD8+	cluster of differentiation 8
Ct	Threshold Cycle
DNA	Deoxyribonucleic Acid
dsDNA	Double-Stranded DNA
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme- Linked Immunosorbant Assay
FR	Free radical
GGT	Gamma-glutamyl transferase
HAV	Hepatitis A Virus
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HCC	Hepatocellular carcinoma
HIV	Human immunodeficiency virus
IC	Internal control
INR	International normalized ratio
IU/L	International units/liter
MDA	Malondialdehyde
NK	Natural killer
n M	Nano meter
PCV	Pack cell volume
PT	Prothrombin time
QRT-PCR	Quantitative Polymerase Chain Reaction
RNA	Ribonucleic acid

ROS	Reactive Oxygen Species
RT-PCR	Real Time -Polymerase Chain Reaction
SD	Standard deviation
SOD	Superoxide dismutase
SPSS	Statistical Packages for Social Sciences
V.C	Vitamin C
WHO	World Health Organization
US	United state

Chapter One

Introduction and Literature Review

Chapter One

Introduction and Literature Review

1.1 Introduction:

Viral hepatitis is a systemic disease, including the liver as the major site for viral replication cycle [1]. Many viruses can cause hepatitis like A, B, C, D, E [2]. Hepatitis B may cause acute (AHB) or chronic hepatitis (CHB) [3], and those with CHB have elevated complications involving cirrhoses and liver cells carcinoma [4]. Hepatitis B virus is a causative agent of significant infectivity and death around the world [5], at which two billion of human beings have been infected with HBV and about 280 million suffering from CHB [6], while 600,000 patients pass through AHB and CHB yearly [7]. According to global surveys, more populations die in a day from CHB than AIDS in 1 year, and nearly 1 million of peoples die from HBV related viral illnesses [5]. Iraq is among the intermediate HBV endemic countries because the infected rate of HBV is between (3%-4.5%) among the population and (2%-3%) among apparently healthy blood donors [8].

Chronic HBV infection is dangerous because there are often no symptoms (even liver blood tests may be normal). As many as 2 out of 3 chronically infected persons are not aware they have been infected. For this reason, the Hepatitis B virus is a silent killer [9]. Detection of serological markers is the mainstay of diagnosis of HBV infection and the most reliable marker of HBV carriage is HBV surface antigen (HBsAg) in serum [10]. HB envelope antigen (HBeAg) is generally used as a secondary marker to indicate infectivity of the virus. The minority of chronic HBV carriers in whom HBeAg can be detected have a particularly high risk of progressive liver disease and end-stage liver

failure [11]. The monitoring of hepatitis B virus DNA in serum is as crucial as serological markers in predicting the clinical outcome of infection. More recently, molecular diagnostic methods have been used to quantify the levels of HBV DNA in serum as a marker of viral replicative activity. The detection and quantification of HBV DNA load are reported to have prognostic value for the outcomes of acute and chronic HBV infections [12]. Quantification of HBV DNA may be a more useful measure than HBeAg as genetic variants of HBV may continue to replicate at a high level without secreting HBeAg. Quantification of HBV DNA can be helpful to assess the efficacy of antiviral therapy as a more direct method of detecting viral replication than serologic markers [13]. More recently, most HBV DNA assays use real-time PCR techniques. The development of real-time PCR methodology has further improved the ease with which HBV DNA levels can be monitored and has increased the range over which such levels can be accurately quantified [14].

Oxidative stress (OS) is an imbalance between the free radicals (FR) formation in cells and an antioxidant defense that includes enzymatic and non-enzymatic antioxidant and some trace elements, which are responsible for oxidative damage to proteins, lipids, and nucleic acids, and modifies their structure as well as functioning [15].

Mitochondria contain their scavenging mechanisms for ROS which are essential and needed for the survival of the cell, but when the production of ROS in mitochondria become in high average more than their capacity of scavenging. This leads to incomplete metabolism of 1-3% of depreciated, and the results are free radicals (FR) [16] such as Superoxide ($O_2^{\cdot -}$), Hydrogen peroxide (H_2O_2) and Hydroxyl radical ($OH^{\cdot}$) [17].

Free radicals are produced continuously in all aerobic organisms by the oxygen through intracellular metabolism as a response to an external stimulus. ROS are provided in the cells in many organelles and numerous enzymes, but the ROS more significant source is the electron transport chain in the mitochondria [(18)].

Lipid, when occur, will give the end products is called malondialdehyde, which is most important lipid peroxidation metabolite [(19)]. Malondialdehyde is not the toxic compound product that is found at low physiological concentrations in its normal state, but high concentration associated with the pathological condition and these will result in damage of DNA [(20)].

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Oxidative damage has been reported to be involved in several hepatic diseases [(21)]. Antioxidant systems are neutralizing the harmful effects of the endogenous Reactive Oxygen Species (ROS) products. Under certain conditions, the oxidative or anti-oxidative balance shifts towards the oxidative status as a result of the increase in ROS and/or impairment in an antioxidant mechanism [(22)]. The antioxidant action of non-enzymatic antioxidants balanced the harmful effects of oxidative damage in addition to antioxidant enzymes. In spite of the presence of antioxidant defense system of the cells to counteract oxidative damage from reactive oxygen free radical species, oxidative damage accumulates during the life cycle and it is capable of direct oxidative damage to macromolecules [(23)].

Cytokines represent a large family of molecules, including chemokines, interleukins (ILs), interferons (IFNs) and members of the tumor necrosis factors (TNF) family; all which play an essential role in the initiation and regulation of immune response. Therefore, the main

biological mediators in the immune response are cytokines, which are low molecular weight-peptides produced by a large variety of cells that have a broad range of physiological functions, and accordingly, it has been highlighted that cytokines might affect susceptibility to and/or natural course of HBV infections [24].

In Iraq, several studies on HBV prevalence and genotyping were carried out. In Anbar Province, to our simple knowledge there is no previous study dealing with molecular detection and characterization to oxidative stress of HBV, and study the role of some interleukins and their effects, therefore this study was conducted for molecular detection of HBV and determination the oxidative stress and effect of some interleukin and liver function test and hematological parameters among our patients who were clinically suspected as having hepatitis.

1.2 Literature Review:

1.2.1 The Liver:

The liver is a vital organ and is the largest internal organ in the body, constituting about 2.5% of an adult's body weight. It has a wide range of functions, including glycogen storage, protein synthesis, detoxification, red cell destruction, hormone production, and bile production for lipid emulsification [25]. It is located in the right upper quadrant of the abdomen below the right lower rib cage against the diaphragm and extends variably to the left upper quadrant [26]. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions [27].

1.2.2 Liver diseases:

Liver disease is defined as any change in the anatomy or function of the liver. The liver is one of the principal organs of the body which has a tremendous capacity to detoxify toxic principle and synthesize useful principles. Thus, damage to the liver can affect our body tremendously (28). There are over 100 different forms of liver disease that affect men, women, and children; they include Wilson's disease, hepatocellular carcinoma, liver cirrhosis, alcoholic liver disease, non-alcoholic fatty liver disease, jaundice, and hepatitis (29).

1.2.2.1 Hepatitis:

Hepatitis is a general term that means inflammation of the liver. Damaged hepatic cells are often repaired in 8 to 12 weeks, or may develop to cirrhosis and fibrosis. There are many reasons for the liver to be inflamed by viral, toxic, metabolic, pharmacologic, or immune-mediated attack on the liver (30) (31).

1.2.2.1.1 Viral Hepatitis:

Viral hepatitis is an infectious disease that refers to the inflammation of the liver that can evolve towards cirrhosis or liver cancer. It is a severe health burden worldwide. To date, five viruses are known to cause hepatitis: A, B, C, D (or delta), and E. These viruses can be differentiated by many criteria, including mode of transmission, incubation period, and potential for becoming chronic infections (25) (29).

Viral Hepatitis may occur with limited or no symptoms but often leads to jaundice, anorexia (poor appetite) and malaise. Viral Hepatitis can be divided into two subgroups according to its duration:

- Acute viral hepatitis – lasting less than six months

- Chronic viral hepatitis – lasting longer than six months [31] [32].

1.2.2.1.1.1 Hepatitis B Virus (HBV):

Hepatitis B is a potentially life-threatening liver infection caused by a specific type of virus called hepatitis B virus (HBV) [33]. It is estimated that 40% of the world's population has had contact with or are carriers of the hepatitis B virus (HBV) [27]. HBV infection is a major cause of chronic liver diseases, such as liver fibrosis, cirrhosis, and even hepatocellular carcinoma (HCC) [34]. This corresponds to an estimated 350-400 million HBV carriers. Thus, HBV infection is one of the most important infectious diseases worldwide [35]. Around one million persons die of HBV-related causes annually. There is a wide range of HBV prevalence rates in different parts of the world [36]. HBV prevalence varies from 0.1% up to 20%. Low prevalence areas (< 2%) represent 12% of the global population and include Western Europe, the United States and Canada, Australia and New Zealand. Intermediate prevalence (2-7%) with a lifetime risk of infection of 20-60% and includes the Mediterranean countries, Japan, Central Asia, the Middle East, and Latin and South America, representing about 43% of the global population. High prevalence areas (>8%) include Southeast Asia, China, and sub-Saharan Africa, where a lifetime likelihood of infection is greater than 60%. [10]. Even though the availability of an effective anti-hepatitis B vaccine and advanced therapy, infection of this virus is still a major health challenge throughout the world [37]. Hepatitis B may acquire HBV infection through horizontal transmission via minor breaks in the skin or mucous membranes or close bodily contact [38]. Also, HBV can survive outside the human body for a prolonged period; as a result, transmission via contaminated household articles such as toothbrushes, razors, and even toys [39]. Although HBV DNA detects in various bodily secretions

of hepatitis B carriers, there is no firm evidence of HBV transmission via body fluids other than blood [40].

1.2.2.1.1.2 Morphology of HBV:

The electron microscopic studies and the Immune Fluorescence shows that HBV is a complex particle of 42-44 nm diameter covered with double layer and inside it, there's a nucleocapsid which surrounds the DNA [10], (Fig. 1-1).

The antigen in the virus core s referred to as the core antigen (HBcAg) which only exists in the hepatocytes during the acute infection of HBV. The antibodies against this antigen generated early during the acute phase of the disease and shortly after the symptoms appearance of hepatitis [38], whereas the surface Antigen of the virus is called surface antigen of hepatitis B virus (HBsAg). This antigen has three phenotypes:

- The preS1 (or large): a Big spherical particle with 42 nm diameter known as Dann's particle represents the virus itself and its existence shows the infection with the virus.
- The preS2 (or middle): Roundy particle with 22 nm in diameter.
- The S (or small): a Tubular particle with 22 nm in diameter and long 50-200 nm [41].

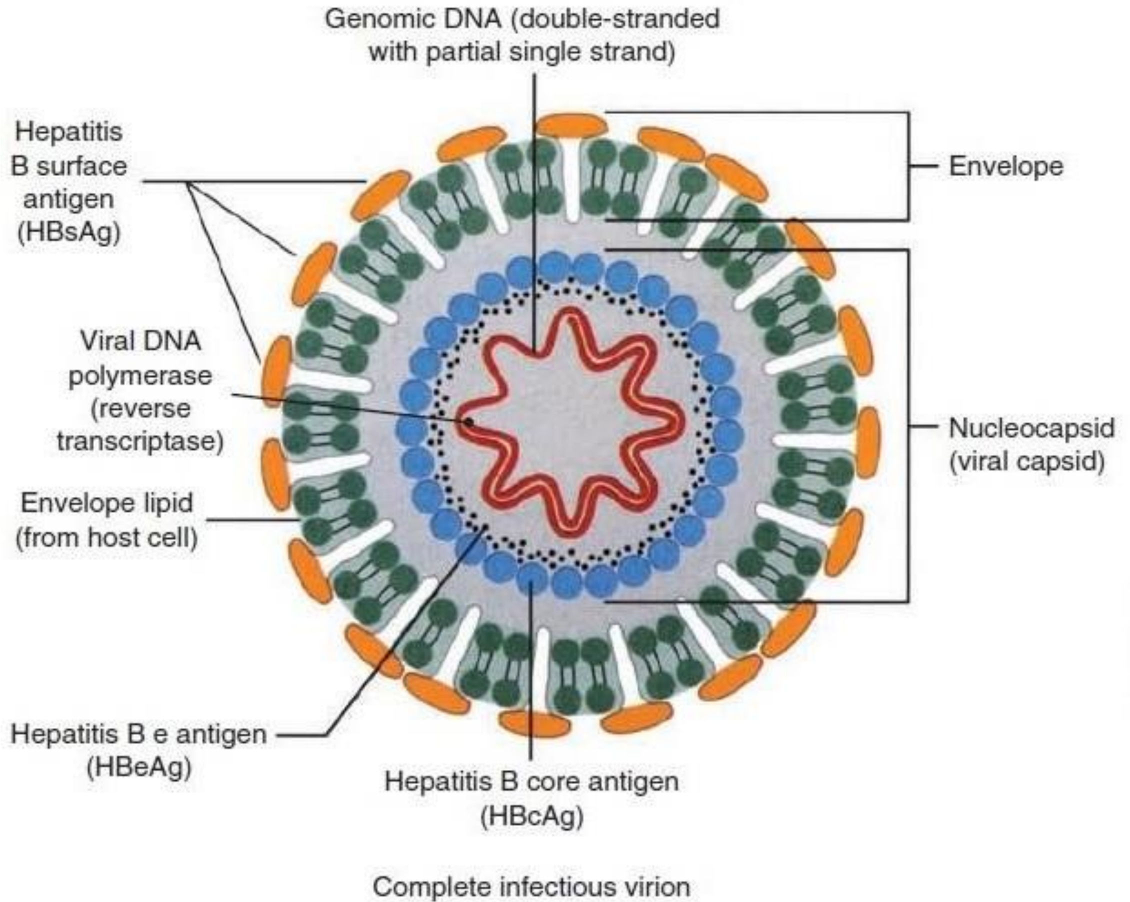


Figure 1-1: Diagram of hepatitis B virion [42].

1.2.2.1.1.3 Clinical types of HBV infection:

A- Silent Primary Infection

In healthy immune-competent individual HBV infection often run as subclinical course and leads to immunity [43]. Asymptomatic cases can e identified by detecting biochemical or virus-specific serologic alterations in their blood, and they may become silent carriers of the virus and constitute for further transmission to others [44].

B- Acute HBV Infection

The incubation period of acute hepatitis B (AHB) infection is asymptomatic in about 90% of cases because the number of HBV-infected hepatocytes is still small at the time when an efficient immune

defense s initiated, so the infection is self-limited and remains without symptoms [45].

His spontaneously resolves maybe after 4-8 weeks of illness; most patients recover without significant consequences and recurrence [46]. In patients with clinical disease, the onset is usually insidious with tiredness, anorexia, vague, abdominal discomfort, nausea, and vomiting. Sometime arthralgias and rash are often progressing to jaundice, and fever may be absent or mild [45] [10].

C- Chronic HBV Infection

Chronic hepatitis B CHB is a prolonged more than six months of infection. Although most adult patients recover completely from an AHB infection, in a significant proportion 5-10 %, the virus persists in the body, but this figure is much higher in children and 70-90 % of infants infected in their first few years of life become chronic carriers of HBV [46], and 40-70 % of HBV infections before the age of 3 years result in CHB infection [45]. The associated inflammatory liver disease is variable in severity. It is always much milder than in AHB, but it can last for decades and proceed to cirrhosis, and it's associated with a 100-fold increase in the risk of developing an HCC [46]. Three phases of viral replication; high, low, and non-replicative, occur during CHB infection, the risk of death from cirrhosis and HCC is 15-25 % [47]. A CHB infection is believed to be common in Iraq as well as in many other developing countries [48].

D- Fulminant HBV infection

Fulminant hepatitis B (FHB) is a rare condition that develops in about 1% of Cases; it is caused by massive necrosis of liver substance and is usually fatal [46]. Genetic heterogeneity of HBV, co-infection or superinfection with other viral hepatitis agents, or host immunological factors may associate with the development of FHB [47]. A rapid fall in

Alanine transferase (ALT) and aspartate transferase (AST) in patients with fulminant hepatic failure may be erroneously interpreted as resolving the hepatic infection when infecting hepatocytes are being lost, and the outcome is fatal. Survivors of FHB is rarely become infected persistently, and HBsAg carriers frequently have no history of recognized acute hepatitis [49].

E- Occult hepatitis B

Occult (Hidden) hepatitis B is defined by the presence of HBV-DNA in serum or liver in the absence of HBsAg [50]. Serum HBV-DNA level is usually less than 10^4 copies/ml. Occult HB has been found in patients with HCC, past HBV infection, or chronic HCV, and individuals without serological markers [47]. The frequency of the diagnosis depends on the relative sensitivity of HBV-DNA assays and the prevalence of HBV infection in the population. Collectively, around 30- 35% of HBsAg-negative subjects with CHB infection with or without HCC have positive serum HBV-DNA range 5-55 % [51] [52]. The prevalence of HBV-DNA is higher in anti-HBc-positive, but anti-HBs-negative patients, ranging from 7 % to 60 % in populations highly exposed to HBV [25]. The clinical significance of occult HBV infection remains unclear. Also, occult HBV infection has been associated with cryptogenic CH and HCC[52].

1.2.2.1.1.4 Laboratory diagnosis of hepatitis:

The detection of hepatitis B virus infection involves serum or blood tests that detect either viral antigens (proteins produced by the virus) or antibodies produced by the host. Interpretation of these assays is included [10]. The hepatitis B surface antigen (HBsAg) is most frequently used to screen for the presence of this infection. Individuals who remain

HBsAg positive for at least six months are considered to be hepatitis B carriers. Carriers of the virus may have chronic hepatitis B [38].

1.2.2.1.1.5 Markers of Hepatitis B and its significance to diagnostic criteria:

Serological testing to diagnose HBV infection involves the measurement of a variety of distinct HBV specific antigens and antibodies that the host reacts to these antigens after initial HBV infection [53].

A- Hepatitis B Surface Antigen (HBsAg):

HBsAg is the first viral antigen to be detected appearing in plasma of patients with acute HBV infection before symptoms appear [53]. The incubation of the Hepatitis B Virus (Hepatitis B) (time from the acquisition of HBV to the onset of clinical symptoms) is typically between 8 to 12 weeks [54].

The first serologic marker to appear is hepatitis B surface antigen (HBsAg), which can initially be detected in serum from 1 to 12 weeks (average, 30 to 60 days) after infection [55]. The HBsAg level increases when symptoms appear and decrease after 2-3 months [54]. The presence of HBsAg in plasma proves the presence of HBV DNA virus in hepatocyte, and Testing HBsAg is an indicator of HBV infection, the presence of HBsAg for more than six months generally indicates chronic HBV infection [56]. HBsAg is not detectable in patients with resolved HBV infection [57].

A negative test for HBsAg in some acute HBV infectious patients might suggest that the current assay does not detect a shallow level of HBsAg or HBsAg is neutralized by anti-HBs antibodies [55].

B- Hepatitis B envelope Antigen (HBeAg)

HBeAg develops one week after HBsAg is detectable; HBeAg usually disappears about three weeks before HBsAg disappears [53]. The presence of HBeAg in the serum of patients indicates a chronic HBV infection [54]. The presence of HBeAg generally correlates with a higher degree of infectivity [53]. Therefore, HBeAg-positive patients are potential HBV carriers to transmit the disease to others because the presence of HBeAg means that HBV is replicating [56]. The risk of perinatal transmission of HBV is about 85-90% if the mother is both HBsAg-positive and HBeAg –positive [57].

C- Hepatitis B Core Antigen (HBcAg)

The HBcAg is an intracellular antigen synthesized within infected hepatocytes. HBcAg is not detectable in plasma. Anti-HBc antibodies can be detected in the sample of hepatocytes taken after a liver biopsy due to immunization upon sampling [54].

D- Total Hepatitis B Core Antibody (Total Anti-HBc)

The first antibodies are classified as IgM and IgG and generally appear after the appearance of HBsAg [58]. The primary detectable antibodies to appear around eight weeks after infection with HBV are antibodies to HBV core protein [37]. Anti-HBc IgM is present in the first weeks of the disease indicating current HBV infection; Anti-HBc IgG appears later and persists longer [57]. Anti-HBc may continue months to years in the convalescent period after acute HBV infections and persist longer in chronic HBV infections [56]. Antibodies to HBcAg do not neutralize the virus and anti-HBc is not protective against HBV re-infection [54].

E- Hepatitis B envelope Antibody (Anti -HBe)

Anti-HBe is usually detectable between 12 and 16 weeks, when HBeAg disappears [58]. Anti-HBe is not detectable until the immune

system has cleared most of the HBe antigens from the blood. The presence of anti-HBe generally indicates an excellent immune response to HBV infection [54].

F- Anti-HBs Ag

Anti-HBs antibodies appear after three-month of infection with HBV and generally at that time HBsAg disappears [56]. Anti-HBs neutralize the HBsAg and is protective for re-infection. IgM anti-HBs is present in the acute period; IgG anti-HBs appears later and persists longer [57]. The presence of anti-HBs is an indicator of recovery. Anti-HBs play an essential role to protect patients from HBV re-infection; therefore, anti-HBs is a component to be used to produce HBV hyper-immune plasma [58]. When vaccinated with HBV vaccine, anti-HBs is the only antibody present in the bloodstream [54].

1.2.2.1.1.6 Hepatitis B DNA (HBV DNA):

HBV DNA can be detected very early after HBV infection and generally indicates active viral replication [58]. The presence of HBV DNA is direct evidence of HBV in the bloodstream. A quantitative test of HBV DNA can be used as an indicator of disease progression [56] [10].

1.2.2.1.1.6.1Quantitation Real-time PCR (qRT-PCR).

The real-time PCR technique is one of the emerging technologies that, although only described for the first time about a decade ago, have become the method of choice for quantification of DNA and RNA levels in cells, tissues and tissue biopsies [59]. Quantitation Real-time PCR this method, the target amplification, and detection steps co-occur. These methods require individual thermal cyclers that can monitor the fluorescence emission from the sample. The computer software supporting the thermal cycler monitors the data at every cycle and generates an amplification plot for each reaction [60].

This method identifies both the HBV genotype and the DNA level in serum, which aids in the prediction of therapeutic outcome .by using SYBR green I (a nucleic acid dye that binds to double-stranded DNA) or fluorescent probes allows the detection and quantitation of HBV DNA [(61)]. It is well known that melting curve analysis enables the determination of genotypes. Melting temperature (Tm) value differs between different genotypes depending on the complementarily between probe and target and/ or GC content of the hybridization sequence [(62)]. Unlike other methods for genotyping, real-time PCR does not allow for cross-contamination, is time-saving, has a high throughput, and is highly sensitive [(61) (63)]. The main disadvantage of this method is that it has a lower ability to distinguish between genotypes with proximity between their Tm values, such as A and C, and thus could not be applied in countries such as North America, which include different genotypes [(63) (64)]. Real-time PCR is extremely useful for quantification of viruses and bacteria, and to a lesser extent also for fungi, parasites, and protozoans. Most of the assays described in literature allow an increased sensitivity and enhanced speed of microbial detection as compared to the classically used culture techniques [(59)].

Finally, detection and quantitation of amplification products can be carried out with molecular beacons. Real-time PCR decrease the time required to perform nucleic acid assays because there are no post-PCR processing steps. The main advantages of these methods are also the decrease in contamination and the possibility for quantitative applications [(60)].

1.3 Liver Function Tests as biochemical markers:

The assessment of the severity of liver disease should include biochemical markers, such as aspartate aminotransferase (AST) and

ALT, gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, prothrombin time and serum albumin, blood counts, and hepatic ultrasound. Usually, ALT levels are higher than AST. However, when the disease progresses to cirrhosis, the ratio may be reversed [(10)]. Once an individual has been diagnosed with chronic HBV infection, follow-up testing must be performed for alanine aminotransferase (ALT), a marker of liver cell inflammation. Repeat periodic testing is indicated because the ALT levels can fluctuate (e.g., from less than the upper limit of normal to intermittently or consistently elevated). Sustained and intermittent elevations in ALT beyond the upper limit of normal are indicative of hepatic inflammation and correlate with an increased risk of progressive liver disease [(41)] [(21)]. A gradual decline in serum albumin concentrations and prolongation of the prothrombin time, often accompanied by a drop in platelet counts, are characteristically observed once cirrhosis has developed [(38)].

1.3.1 Tests for the liver's ability to transport:

The tests include serum bilirubin, bromsulphalein [BSP], indocyanine green (ICG), serum bile acids, serum caffeine, serum lidocaine metabolites, and breath tests. Each of these tests measures the ability of the liver to clear endogenous or exogenous substances from the circulation [(65)].

1.3.1.1 Serum bilirubin:

Bilirubin is the orange-yellow pigment derived from senescent red blood cells. It is extracted and biotransformed mainly in the liver, and excreted in bile and urine. The overall chemical structure of bilirubin establishes by X-ray crystallography [(28)].

Hyperbilirubinemia has seen in viral hepatitis, hepatocellular damage, toxic or ischemic liver injury and which is directly proportional to the degree of histological injury of hepatocytes [66] [67]. Total bilirubin increase in hepatitis B and hepatitis C [68] [69].

1.3.2 Tests to detect damage to hepatocytes:

These include the entire enzyme tests, of which the aminotransferases and alkaline phosphatase are the most commonly ordered and the most useful [65].

1.3.2.1 Alanine aminotransferase and aspartate aminotransferase (ALT&AST):

Alanine transaminase or ALT is a transaminase enzyme (EC 2.6.1.2). It is also called serum Glutamic pyruvic transaminase (SGPT).

Alanine transaminase is found in serum and various bodily tissues but is most commonly associated with the liver. It catalyzes the two parts of the alanine cycle. It catalyzes the transfer of an amino group from alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate. It is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health. When used in diagnostics, it is almost always measured in international units/liter (U/L) [70]. Significantly elevated levels of ALT (SGPT) often suggest the existence of other medical problems such as viral hepatitis [71] , diabetes, congestive heart failure, liver damage, bile duct problems [70] [72].

Aspartate transaminase (AST), also called aspartate aminotransferase or serum Glutamic oxaloacetic transaminase (SGOT) is a pyridoxal phosphate (PLP)-dependent transaminase enzyme (EC 2.6.1.1). Aspartate transaminase catalyzes the reversible transfer of α-

amino group between aspartate and glutamate and, as such, is an essential enzyme in amino acid metabolism. Aspartate transaminase is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health[73].

Aspartate transaminase catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate [28]. Aspartate transaminase is similar to (ALT) in that both enzymes are associated with liver parenchymal cells. The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle, while AST is located in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells [74]. As a result, ALT is a more specific indicator of liver inflammation than AST, as AST may also be elevated in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma. Aspartate transaminase was defined as a biochemical marker for the diagnosis of acute myocardial infarction in 1954. However, the use of AST for such a determination is now redundant and has been superseded by the cardiac troponins. Aspartate transaminase is commonly measured clinically as a part of diagnostic liver function tests, to determine liver health [75]. Hepatic diseases may lead to an increase in the serum concentration of aminotransferases, (Both AST and ALT). So, any increase in activity of ALT enzyme in the serum of patients is regarded as a specific marker for liver damage [76].

In many cases of acute viral hepatitis, aminotransferase levels (AST), usually reach the peak concentration before the appearance of

jaundice and then decrease gradually [77] [76]. Increased liver enzymes such as (GPT), (GOT) and (ALP) in both patients with hepatitis B [74], B and C [78].

1.3.2.2 Alkaline phosphatase (ALP, EC 3.1.3.1):

Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called dephosphorylation [79]. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. It is sometimes used synonymously as an essential phosphatase. These enzymes are widely distributed in the body, but the main sites of production are the liver, gastrointestinal tract, bone, placenta, and kidney [80]. Alkaline phosphatases are post-translationally modified, resulting in the production of several different isoenzymes which differ in abundance in different tissues. Alkaline phosphatase enzymes in the liver are located in cell membranes of the hepatic sinusoids and the biliary canaliculi. Accordingly, levels rise with intrahepatic and extrahepatic biliary obstruction and with sinusoidal obstruction, as occurs in infiltrative liver disease [81].

Moreover, any damage to hepatocytes may lead to the release of large amounts of ALP into the serum of patients [76]. Elevated levels of ALP may be seen in cirrhosis, hepatitis and congestive cardiac failure [82].Increased serum ALP in hepatitis B [74] [78].

1.3.2.3 *γ*- glutamyl transferase (GGT, EC 2.3.2.2):

Gamma-glutamyltransferase is an enzyme that catalyzes the transfer of a γ -glutamyl group from glutathione (GSH) and other γ-glutamyl compounds to amino acids or dipeptides and water [83], this reaction

produces cysteinyl-glycine moieties, which are always taken within the intracellular milieu by the action of membrane di-peptidases as precursors for glutathione resynthesize as shown in Scheme (1.2) [(84)].

It also catalyzes the hydrolysis of the c-glutamyl bond [(85)]. It is present in the cell membrane of nearly all human cells. It is most abundant in the kidney, liver, pancreas, and intestine, but the majority of the GGT detected in serum derives from the liver. GGT is the most sensitive biomarker of hepatobiliary disease. GGT is indicated in the following infection (e.g., viral hepatitis and other specific infections such as amebiasis, tuberculosis, psittacosis, and similar infections) [(86)].

GGT activity is elevated in cholestatic liver disease, alcoholic, and another fatty liver disease, and can be induced by many drugs, including barbiturates and phenytoin [(87)]. High serum GGT levels may be considered an indicator of an advanced stage of fibrosis in patients diagnosed with chronic viral hepatitis B [(88)].

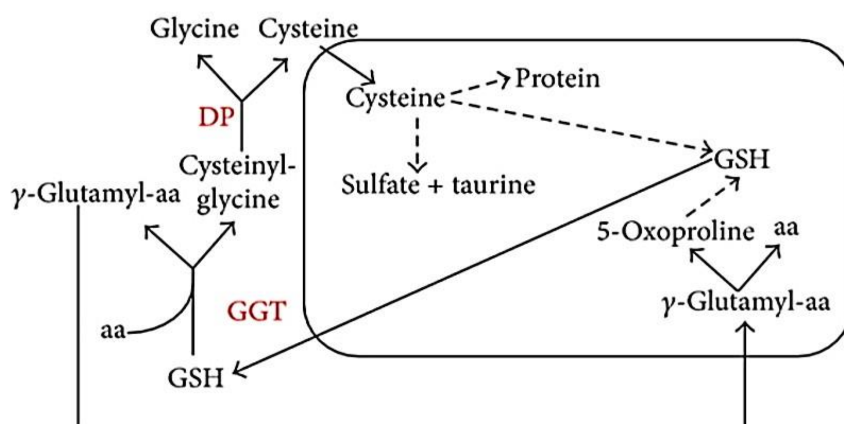


Figure 1.2 Schematic diagram of γ-Glutamyl cycle [(89)].

1.3.3 Tests of the biosynthetic capacity of the liver:

These groups include serum albumin, Total serum protein, ceruloplasmin, ferritin, α1-antitrypsin, lipid profile, and blood-clotting factors. These substances are synthesized in the liver for transport into the circulation [(65)].

1.3.3.1 Total protein:

Total Serum protein, also called total plasma protein or total protein, is a biochemical test for measuring the total amount of protein in blood plasma or serum. Protein in the plasma is made up of albumin and globulin which are the main two groups of the protein. The globulin, in turn, is made up of α1, α2, β, and γ globulins. These fractions can be quantities using protein electrophoresis [65]. Proteins are substances which consist of smaller building blocks called amino acids and are essential components of all cells and tissues. Total protein is considered as the most abundant compounds in serum. The proteins are involved in enzymes, hormones, and antibodies as well as osmotic pressure balance. The possible etiology of decreased serum total protein secondarily decreased synthesis of protein by the liver [90]. Total protein may increase in hepatitis B and C [68].

1. 3.3.1.1 Albumin:

Albumin is the major protein that circulates in the blood and is made by the liver. It can be used as a supplementary test for hepatic biosynthetic functions. Increased albumin may be due to dehydration or improper use of protein whereas decreased levels are common in infections, liver diseases, ulcers and some kidney diseases [91]. A low serum albumin concentration refers to liver impairment. It is observed that decreased level of albumin is not seen in the acute failure of the liver because it takes several weeks of impaired albumin production until the serum albumin level drops. The most important cause for low albumin level is chronic liver failure caused by cirrhosis. The serum albumin concentration is usually normal in chronic liver disease until cirrhosis, and significant liver damage develops [92].

1. 3.3.1.2 Globulins:

Globulin is one type of protective antibody produced by immune system that helps identify and fight infections. Increased globulin levels may be due to chronic inflammation, kidney infection, stress, liver diseases and parasite infestations whereas decreased globulin may be due to anaemia, low hydrochloric acid and depressed immune systems [(67)]. The estimation of total proteins in the body is helpful in differentiating between a normal and damaged liver function as the majority of plasma proteins like albumins and globulins that are produced in the liver. Total protein is often reduced slightly but the albumin to globulin ratio shows a sharp decline during hepatocellular injury [(93)].

1.3.3.2 Prothrombin Time (PT)

The liver forms the blood substances used in coagulation, substances formed in the liver that is used in the coagulation process. These include fibrinogen, prothrombin, accelerator globulin, factor VII and several other relevant factors. The metabolic processes of the liver require vitamin K for the formation of several of these substances, mainly prothrombin and factors VII, IX and X in the absence of vitamin K the concentration of all these decreases markedly and these almost prevents blood coagulation [(28)]. The liver is involved in clearing some of the clotting factors from serum. The levels of components of the clotting mechanism are frequently abnormal in the course of the hepatic disease. These abnormalities can be assessed with tests in which one factor or the interplay of many factors is measured. The one-stage prothrombin time of quick is one of the most useful tests available. It is used to measure the rate at which prothrombin is converted to thrombin. This occurs in the presence of a tissue extract (thromboplastin), calcium ions, and a series of activated coagulation factors (factors I, II, V, VII, and X), and

is followed by the polymerization of fibrinogen to fibrin by thrombin [94]. The prothrombin time is the time it takes plasma to clot after addition of tissue factor (obtained from animals such as rabbits, or recombinant tissue factor, or from brains of autopsy patients). This measures the quality of the extrinsic pathway (as well as the common channel) of coagulation. The speed of the extrinsic pathway is significantly affected by levels of functional factor VII in the body. Also, poor factor VII synthesis (due to liver disease) or increased consumption (in disseminated intravascular coagulation) may prolong the PT. The prothrombin test is not a sensitive index of chronic liver disease, and the prothrombin test is particularly important in the treatment of patients with liver disease. It allows assessment of the tendency to bleed before any contemplated surgical or diagnostic procedure, such as a closed liver biopsy [65].

1.3.3.3 Fibrinogen

Fibrinogen, or factor I, is a blood plasma protein that's made in the liver. Fibrinogen is one of 13 coagulation factors responsible for normal blood clotting [95]. Fibrinogen is involved in both primary and secondary hemostasis, playing an essential role in platelet aggregation and the establishment of a fibrin network [96]. The liver plays a crucial role in hemostasis regulation. The liver parenchymal cells produce most of the factors and inhibitors in the clotting and fibrinolytic systems, and the liver greatly aids in clearing activated clotting or fibrinolysis enzymes from the circulation, which protect against both hemorrhage and inappropriate activation of coagulation [97].

Blood coagulation is a process in which several components of the blood from a clot. When blood escapes from a rupture in a blood vessel, coagulation is triggered. Several proteins, called coagulation factors, go into action to produce thrombin. The thrombin then converts fibrinogen

to fibrin. Fibrin produced from fibrinogen is the main protein in a blood clot. It surrounds the cells in the blood and plasma and helps form the clot. The resulting clot, which is stabilized by Factor XIII, remains intact from 10 to 14 days, the time required for healing to take place. When there is a problem with fibrinogen, i.e., either it is missing, or it does not function properly, the clot has difficulty forming. This can result in hemorrhaging or thrombosis [98].

There is an alteration in the coagulation factors in hepatitis. The degree of alteration indicates the severity of liver disease [99]. The study suggested that low plasma fibrinogen may be a useful indicator for predicting poor prognosis in Acute-on-Chronic Hepatitis B Liver Failure[97].

1.4 Oxidative stress:

Oxidative stress is defined as an imbalance between the production of free radical and oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms referred to as antioxidants. This Inequality leads to the damage of important biomolecules and cells [100]. The harmful effects of ROS are balanced by antioxidants, some of which are enzymes present in the body. Despite the presence of the cell's antioxidant defense system to counteract oxidative damage from ROS, oxidative damage accumulates during the life cycle leading to cause diseases, aging and age-dependent diseases such as cancer, neurodegenerative disorders, cardiovascular disease and other chronic Conditions [101]. Hepatitis B is associated with increased levels of oxidative stress [102] [103]. Oxidative stress plays a crucial role in several disorders including aging, cancer, diabetes, Alzheimer's, strokes, viral infections, neurodegenerative processes (including cell death, motor neuron diseases and axonal injuries and infraction, and brain edema [104].

1.5 Free radicals:

Free radicals are highly reactive atoms or molecules with one or more unpaired electron(s) in their external shell and can be formed when oxygen interacts with certain molecules. These radicals can be produced in cells by losing or accepting a single electron, therefore, behaving as oxidants or reductants [105].

Free radical species are unstable and highly reactive. They become stable by acquiring electrons from nucleic acids, lipids, proteins, carbohydrates or any nearby molecule causing a cascade of chain reactions resulting in cellular damage and disease [106]. These are formed as natural by-products of oxygen metabolism and serve the purpose of burning bacteria and refuse body matter but when out of control, they become toxic and start damaging body tissues by a process called oxidative stress [107]. These are two major types of free radical species: reactive oxygen species (ROS) and reactive nitrogen species (RNS) [108]. As shown in the table (1-1).

Table 1-1 Reactive oxygen species and Reactive nitrogen species in the human body [109].

Type	Radical	Non Radical
Reactive Oxygen Species (ROS)	Superoxide $O_2^{\cdot -}$ Hydroxyl $^{\cdot}OH$ Peroxyl $RO_2^{\cdot}$ Alkoxyl $RO^{\cdot}$ Hydroperoxyl $HO_2^{\cdot}$	Hydrogen peroxide H_2O_2 Hypochlorous HOCl Ozone O_3 Singlet oxygen $^1O_2$ Hypobromous HOBr
Reactive Nitrogen Species (RNS)	Nitric oxide $NO^{\cdot}$ Nitrogen dioxide $NO_2^{\cdot}$	Nitrous acid HNO_2 Nitrosyl cation NO^+ Nitrosyl anion NO^- Dinitrogen tetroxide N_2O_4 Dinitrogen trioxide N_2O_3 Peroxynitrite NO_3^- Nitronium cation $^+NO_2$ Nitryl chloride NO_2Cl Alkyl peroxy nitrites ROONO

The reactive oxygen species (ROS) is a collective term which includes both oxygen radical and specific nonradicals that are oxidizing agents and/or quickly converted into radicals [110]. Reactive nitrogen species (RNS) consists of both nitrogens containing radicals and non-radicals [111]. Radicals are less stable than non-radical species, although their reactivity is generally stronger [106].

Reactive oxygen species cause damage to all major classes of macromolecules in cells. The phospholipids of plasma and organelle membranes are subject to lipid peroxidation, a free radical chain reaction initiated by removal of hydrogen from a polyunsaturated fatty acid by hydroxyl radical. The resulting lipid radicals then react with O_2 a diradical to form lipid peroxy radicals and lipid peroxide along with malondialdehyde, which is water soluble and can be detected in blood[112].

1.5.1 The source of reactive oxygen species (ROS):

1.5.1.1 Endogenous source of reactive oxygen species:

Free radicals (FR) are produced continuously in all aerobic organisms by the oxygen through the intracellular metabolism as the response to an external stimulus. ROS are provided in the cells in many chambers and numerous enzymes, but the ROS greater source is electron transport chain in the mitochondria [113].

Generally, reactive oxygen species that endogenously are generated in the body via four different mechanisms as follow: first; by the normal oxygen metabolism of nutrients. Second; using oxidants by white blood cells to destroy parasites, bacteria, and viruses such as superoxide, nitric oxide, and hydrogen peroxide. Third; others called peroxisomes which are cellular components generate hydrogen peroxide through the degradation of fatty acids and other molecules. Finally, cytochrome P450 is an enzyme in the cells represents one of the primary defenses in the

body against toxic chemicals ingested with food like drugs and pesticides [114].

1.5.1.2 Exogenous sources of reactive oxygen species:

A-Smoking of cigarette:

Cigarette smoking contains several reactive oxygen species, and organic elements included nitric oxide and superoxide .also, cigarette inhalation into lung can stimulate some endogenous mechanisms included aggregation of neutrophils and macrophages which in the further will elevate the oxidant damage [115].

B-Ozone exposure:

Ozone is typically found in the stratosphere zone of atmosphere and is toxic and unstable gas; Ozone layer absorbed ultraviolet radiation and prevented from reaching to the surface of the earth. When ozone depleted the ultraviolet radiation will arrive at earth, little amounts of ultraviolet radiations are necessary for the generation of vitamin D but the increased exposure will results in oxidative stress by producing free radicals [116].

***C-Hypoxia*:**

The term hypoxia is precisely characterized as a state of oxygenation that is below the norm for a particular tissue; this leads to higher production of ROS [117].

De-ionizing radiation:

Ionizing radiation with the oxygen can convert superoxide, hydroxyl radicals, and organic radicals to hydrogen peroxide and the hydroperoxide. Organic hydroperoxides and the hydroperoxide species react with redox-active metal ions included Fe, Cu by Fenton reactions and therefore produced oxidative stress [118].

E-Heavy metal ions:

An ions of heavy metals include iron, arsenic, mercury, cadmium, lead, nickel, and copper. They can consist of the production of ROS and result in cellular injury by enzyme activates depletion during lipoperoxidation and reaction with nuclear proteins [(119)].

1.6 Lipid peroxidation:

It is a complex phenomenon that found in both plant and animal tissues which included the production of lipid radicals (LOO). This mechanism involves consumptions of O2 and rearrangement the double bonds of unsaturated lipids and the final damage of membrane lipids with the liberation of different broken results such as. A ketone, alcohol, aldehydes, alkanes, and others [(120)]. Lipid peroxidation is chain reaction begins with hydrogen removing or addition of oxygen radical and the result is the oxidative injury of polyunsaturated fatty acids [(121)]. The effects of this reaction are falling in membrane fluidity, and the permeability functions of the cellular membrane as well as many products of this reaction such as hydroperoxides and their aldehyde derivatives depress protein synthesis and enzyme activities. In pathological condition, these reactions occur at high rates result in free reactive oxygen and nitrogen species at rates higher than of normal range [(122)].

Lipid peroxidation can be enzymatically occurring by lipid peroxidation enzymes such as lipoxygenases family and non enzymatically occurring by reaction of an FR molecule with poly-unsaturated fatty acids [(123)]. In the first, hydrogen atoms will remove from methyl groups of these lipids producing a lipid radical. In the next, a reaction with molecular oxygen occurs to result in a peroxyl radical. The response of this radical with another poly-unsaturated fatty acid

results in lipid peroxide and a new fundamental starting a chain reaction [124]. Lipid peroxidation, when occur, will give the end products they called is malondialdehyde which is most important lipid peroxidation metabolite [125]. Malondialdehyde is not toxic compound products are found at low physiological concentrations in its normal state, but it's with the pathological condition, and these will result in damage of DNA [20].

1.6.1 Malondialdehyde (MDA):

It is the naturally occurring product of lipid peroxidation [125]. Malondialdehyde is one of most the marker most frequently used to indicate the levels of lipid peroxidation, in the field of exercise, and has been widely used as a biomarker of oxidative stress [101]. Figure (1-3). MDA, a normally can represent the end product of membrane lipid peroxidation, which is one of the most biomarkers which used for free radical mediated injury [126], which can be defined as highly reactive three carbon aldehyde compound produced from polyunsaturated fatty acid peroxidation [20]. Also, it can be released during arachidonic acid metabolism during synthesis of prostaglandins which occur in platelets by cyclooxygenase (COX) and even in the liver cells when prostaglandin endoperoxide is breakdown [127]. Malondialdehyde has the molecular formula (C7H16O4) and its molecular weight 72 Daltons, and the boiling point is 183 Cº and freezing point 130Cº. It is found in plasma at very low levels bound to protein in the normal state but increased in the pathological cases. This compound is not toxic but combined to bases of DNA leads to formation series of products as deoxyadenosine, deoxycytidine, deoxyguanosine and also it targets of nucleotide enzymes of excision repair which may be major endogenous DNA adduct which contributes significantly in the tumor [19].

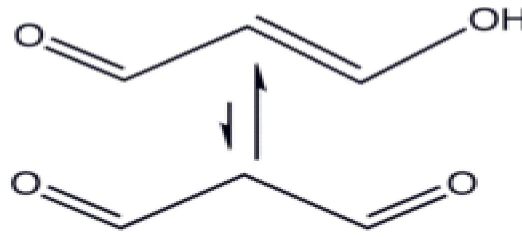


Figure 1-3 Chemical structure of malondialdehyde [128].

1.7 Antioxidants:

Antioxidants are found in the body and their function to neutralize the toxic effects of free radicals [129], the antioxidants can be divided into two groups as follow:

1.7.1 Enzymatic antioxidants:

Enzymes of antioxidants include superoxide dismutase (SOD), glutathione reductase (GSR), glutathione peroxidase (GPX) and catalase (CAT), which can cause reduction of H_2O_2 to water and alcohol [106]. So represent as endogenous source and are active on scavenging the toxic effects of free radicals in mitochondrial membrane [19].

1.7.1.1 Superoxide dismutase (SOD, E.C 1.15.1.1):

Superoxide dismutase is classes of closely related enzymes which are present in almost all cells and the extracellular fluids [130]. They catalyze the breakdown of the superoxide anion (O^{2-}) into hydrogen peroxide (H_2O_2) and oxygen (O_2), and the latter is then mutated to water by glutathione peroxidase and catalase scheme. (1-4). Superoxide dismutase enzymes contain metal ion, cofactors which can be zinc, copper, iron, or manganese depending on the concerned isoenzyme [83]. Superoxide dismutase has three forms: MnSOD located in mitochondria, copper-zinc superoxide dismutase (Cu/ZnSOD) in the cytoplasm, and extracellular superoxide dismutase (EC-SOD). In newborns, the last one is located intracellularly in the cytoplasm, unlike in adults, where it is located

extracellularly, as indicated by its name [131]. Superoxide dismutase is an enzyme that detoxifies superoxide anions and attenuates their toxicities and synergistic detrimental effects on the cells [132]. It is an essential process because superoxide is a dangerous compound (a reactive form of oxygen) [122]. O2·- leak from electron transport chain and damage the cell through mutations in DNA, attack enzymes that make protein and other essential molecules [133]. The role of metal ions is that the active site for these enzymes performs its function in shuffling reaction. Superoxide dismutase floats inside cells to scavenge any superoxide, and its work is promoting and accelerated the reduction of superoxide to form hydrogen peroxide [19].

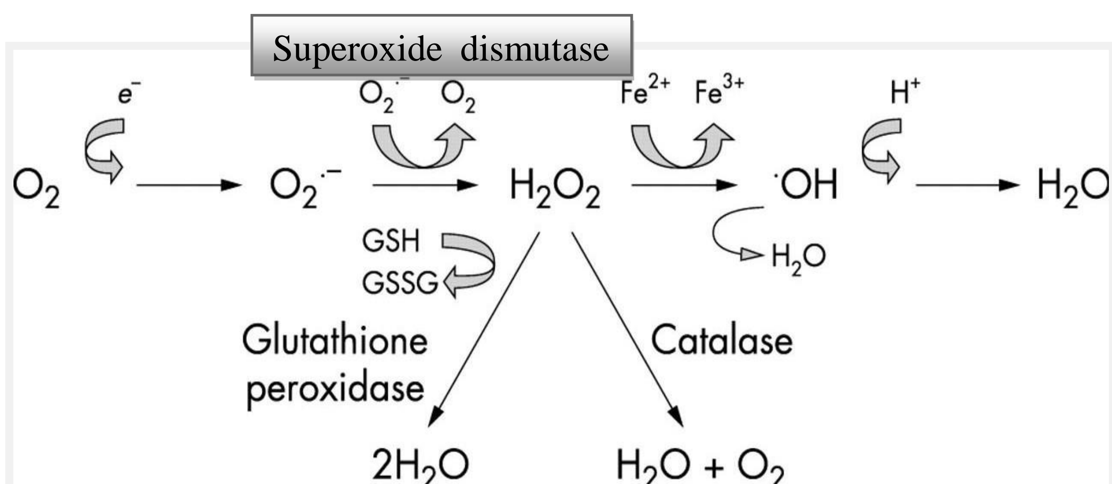


Figure 1-4 Generations of reactive oxygen species [134].

1.7.2 Non-enzymatic antioxidants:

The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants [135].

Metabolic antioxidants, belonging to endogenous antioxidants, are produced by metabolism in the body, such as lipoic acid, glutathione, L-arginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin, etc. [136]. They are the scavengers of ROS and RNS; glutathione inhibits oxidation of membrane lipid, uric acid is the

scavenger of peroxynitrite in plasma, while albumin, bilirubin, melatonin are directly reacted with ROS and form disulfides [132].

Nutrient Antioxidants, belonging to exogenous antioxidants, are compounds which cannot be produced in the body and must be provided through foods or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, and zinc), etc. [133]. Nutrient antioxidants are involved in detoxification of the reactive oxygen species (ROS) and play an essential role in helping endogenous antioxidants for the neutralization of oxidative stress [134].

1.7.2.1 Vitamin C:

Vitamin C or L-ascorbic acid, or simply ascorbate, is an essential nutrient for humans and certain other animal species, vitamin C is a cofactor in at least eight enzymatic reactions including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scurvy [135] [136]. Vitamin C was found in vegetables, fruits (citrus), and pharmaceutical supplements. Vitamin C is a critical factor in human health. This vitamin cannot be created via humans, so, it has been extracted from our diet [137]. (Figure 1-5) Vitamin C is an excellent serum antioxidant because it dissolves in water and vitamin E dissolves in lipids. Both of them are naturally occurring free radical scavengers [64]. The biological role of ascorbate is to act as a reducing agent, donating electrons to various enzymatic and a few non-enzymatic reactions, the one- and two-electron oxidized forms of vitamin C, semidehydroascorbic acid, and dehydroascorbic acid, respectively, can be reduced by the body by glutathione and NADPH-dependent enzymatic mechanisms [138].

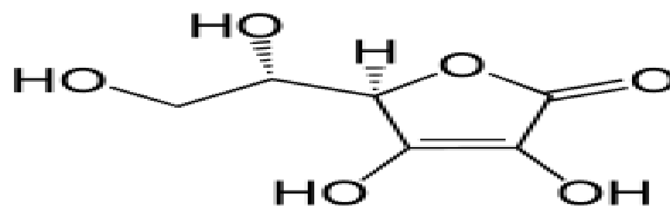


Figure 1-5 Chemical Structure Of Vitamin C [139].

Vitamin C is the most important antioxidants. Vitamin C protection water-soluble substances in the body from damage by free radicals. Free radicals are molecules with an unpaired electron—they are hungry for another electron. Free radicals are unstable and react quickly with other compounds. Usually, free radicals attack the nearest stable molecule, "stealing" its electron [140].

This attack is known as oxidative stress. When the "attacked" molecule-deficient with its electron, it can become a free radical itself, beginning a chain reaction that can continue. If this chain reaction proceeds, it can disrupt a living cell. Free radicals usually occur during metabolism. Also, the body's immune system purposefully creates them to neutralize viruses and bacteria. Other sources of free radicals and other oxidative stresses include environmental factors such as pollution, hard radiation, cigarette smoke, and certain pesticides [138]. Vitamin C is exhibited in that it can neutralize a free radical without becoming a free radical itself. Vitamin C can donate one or two hydrogen atoms to neutralize a free radical. The electrons from the hydrogen atoms which neutralize the free radicals to prevent a free radical chain reaction. After giving two hydrogen atoms with their electrons, vitamin C can generally be reactivated with the addition of two hydrogen atoms [141]. Vitamin C with its two hydrogen atoms is named *ascorbic acid* and is in a state of readiness to perform antioxidant actions. Vitamin C is also called *dehydroascorbic acid* when it has lost its two hydrogen atoms. Vitamin

C is stable both with and without its extra hydrogen atoms [142]. It is not yet sure whether vitamin C and antioxidants can prevent oxidative stress-related diseases and promote health, clinical studies regarding the effects of vitamin C supplementation on lipoproteins and cholesterol have found that vitamin C supplementation does not improve specific disease markers in the blood [143]. Vitamin C may contribute to decreasing risk of cardiovascular disease and strokes through a small reduction in systolic blood pressure. Also, and it was also found to both increase ascorbic acid levels and reduce levels of resistin serum [144].

1.8 Immune system:

The immune system is a system of biological structures and processes within an organism that protects against disease or other potentially damaging foreign bodies [145].To function correctly, an immune system must detect a wide variety of agents, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue, pathogens can rapidly evolve and adapt, and thereby avoid detection and neutralization by the immune system, however, multiple defense mechanisms have also developed to recognize and neutralize pathogens. Even simple unicellular organisms such as bacteria possess a rudimentary immune system, in the form of enzymes that protect against bacteriophage infections [146]. Other basic immune mechanisms evolved in ancient eukaryotes and remained in their modern descendants, such as plants and insects. These mechanisms include phagocytosis, antimicrobial peptides called defensins, and the complement system. Jawed vertebrates, including humans, have even more sophisticated defense mechanisms [147], including the ability to adapt over time to recognize specific pathogens more efficiently. Adaptive (or acquired) immunity creates immunological memory after an initial response to a

particular pathogen, leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination. Disorders of the immune system can result in autoimmune diseases, inflammatory diseases, and cancer [148] [122].

1.9 Inflammation:

Inflammation is a defense strategy against invading agents and harmful molecules that is activated immediately following a stimulus, and involves the release of cytokines and chemokines, which activate the innate immune response [149]. Inflammation is characterized by redness, swelling, heat, and pain, and loss of tissue function, which result from local immune, vascular and inflammatory cell responses to infection or injury. Inflammation is produced by eicosanoids and cytokines, which are released by injured or infected cells. Eicosanoids include prostaglandins that produce fever and the dilation of blood vessels associated with inflammation and leukotrienes that attract specific white blood cells (leukocytes) [150]. Common cytokines include interleukins that are responsible for communication between white blood cells; chemokines that promote chemotaxis; and interferons that have anti-viral effects, such as shutting down protein synthesis in the host cell [151]. Growth factors and cytotoxic factors may also be released. These cytokines and other chemicals recruit immune cells to the site of infection and promote healing of any damaged tissue following the removal of pathogens [152].

1.10 Cytokines:

Cytokines are small molecular weight proteins or peptides messengers between tissues and the immune system and participate in many physiological processes [153].Their principal function is to regulate immune response and initiate autoimmunity. Moreover, they affect the

function of other cells, hematopoiesis, mediate inflammation, wound healing. Cytokines initiate an inflammatory response, by stimulating T and B cells [154]. They are active at deficient concentrations, produced transiently, act locally in the tissue where they are created, focusing on the receptors over the nearby cells [155]. They are released by various types of cells including immune cells like macrophage, T, and B lymphocytes, monocyte, dendritic cells, neutrophils, as well as endothelial cells, fibroblasts, and various stromal cells. Cytokines are the mean of communication between immune and non-immune cells [156]. Cytokines act as specific messengers that enable interactions between cells in both physiological and pathological conditions. Through specific cellular receptors, they regulate the intensity and duration of every stage of the immune response; particularly by modulating the balance between humoral and cell-mediated responses because of their prominent role in immune processes they are often called "hormones of the immune system" or "hormone-like proteins" [157].

A balance between pro-inflammatory and anti-inflammatory cytokines is essential for the development of a well-regulated effector immune response. The overproduction of pro-inflammatory cytokines and/or the deficiency of anti-inflammatory cytokines may lead to immune pathology. They serve as the mediators of cellular differentiation, inflammation, immune pathology, and regulating of immune response as well as apoptosis of the multiple cell types [158].

1.10.1 Types of cytokines:

1- lymphokines: Cytokines made by lymphocytes.

2- Monokines: Cytokines mad by monocytes.

3- Chemokines: Cytokines with chemotactic activities.

4- Interleukins: Cytokines made by one leukocyte and acts on other leukocytes [159].

The liver is a major organ in the production of cytokines. Cytokines are involved in physiologic and pathologic processes occurring in the liver. They play an essential role in liver growth and regeneration, as well as in inflammatory processes including viral liver disease, liver fibrosis and cirrhosis [160]. Cytokines are often produced in a cascade, as one cytokine stimulates its target cells to make additional cytokines, cytokines can also act synergistically (two-or-more cytokines working together) or antagonistically (cytokines causing opposing activities) [161]. Cytokines are key modulators of inflammation, participating in acute and chronic inflammation via a complex and sometimes seemingly contradictory network of interactions. Better understanding of how these pathways are regulated helps facilitate more accurate identification of agents mediating inflammation and the treatment of inflammatory diseases [162].

1.10.1.1 Interleukin 1 (IL-1):

The IL-1 system is a family of polypeptides composed of two agonists (IL-1α and IL-1β), one inhibitor designated IL-1 receptor antagonist (IL-1ra), and two receptors known as IL-1 receptor type I (IL-1RtI) and type II (IL-1RtII) [163]. IL-1α and IL-1β are proinflammatory cytokines produced by monocytes and macrophages. IL-1 production may be induced by the presence of microbial pathogens, bacterial lipopolysaccharides, or other cytokines [30]. Activity of IL-1α and IL-1β is tightly controlled because of its potent and pleiotropic effects on inflammation, and both are expressed at low levels under normal conditions. Transcription, translation, processing, and secretion of IL-1α and IL-1β are all highly regulated processes [164]. However, IL-1α

remains intracellular within monocytes and macrophages and is rarely found outside these cells. IL-1α can be released after cell death and can help attract inflammatory cells to areas where cells and tissues are being killed or damaged [(30)].

IL-1 is essential in inflammatory reactions and regulates the production of Th1/Th2 cytokines. Thus, the invasion, implantation and trophoblasts growth would be affected by this interleukin [(164)]. The result suggests that IL-1receptor antagonist was associated with the development of liver inflammation, which is reduced in patients with hepatitis B-related acute-on-chronic liver failure [(165)].

1.10.1.2 Interleukin-2 (IL-2):

Interleukin-2 was discovered in 1975 as a growth-promoting activity for bone marrow-derived T lymphocytes, and it is coded by a gene located on human chromosome 4 [(166)]. Structurally, IL-2 is a protein consisting of 133 amino acids and has a molecular weight of 15400 Daltons. It is synthesized and released from activated T cells and has a crucial role in cell-mediated immune response, and it was found that IL-2 increases lymphokine release from T, B, and NK cells and has many immunological effects [(167)]. Additionally, it is a potent T cell growth factor that is assumed to amplify lymphocyte responses *in vivo* [(168)]. It is synthesized and released from activated CD4+ T cells, although expression by naive CD8+ T cells, dendritic cells, and thymic cells can also occur [(169)]. Also, it promotes the production of NK-derived cytokines such as tumor necrosis factor alpha (TNF-α), IFN-γ and granulocyte monocyte-colony stimulating factor (GM-CSF), and can act synergistically with IL-12 to enhance NK cytotoxic activity [(166)]. Several forms of IL-2 receptors differ in their affinities for IL-2: a low-affinity receptor consisting of an α chain, an intermediate affinity receptor

comprising of β and γ chains, and a high-affinity receptor consist of all three chains α, β, and γ [168]. The study demonstrated that IL-2 complex therapy could be useful to boost protection against cutaneous virus infection [170]. Elevated levels of interleukin-2 have been shown in the blood of patients with Hepatitis B Virus infection during acute and convalescent phases [171]. Evaluation of serum levels of interleukin-2 in patients with chronic hepatitis [159].

1.11 Role of Cytokines in Viral Hepatitis:

Cytokines play a critical role in the defense against viral infections, both directly, by the inhibition of viral replication, and indirectly, through determination of the common Th1/Th2 pattern of host immune response. However, in the same context of an inflammatory response against a virus, cytokines may also cause liver damage [172]. In acute hepatitis B virus infection, a powerful polyclonal cellular immune response is critical; thus Th1 cytokine release is vital to onset an effective immune response [173]. The cytokines produced by CD4+ and CD8+ cells also play an essential role in down-regulation of HBV replication, demonstrating that it is possible to control a viral infection without the death of infected cells [174]. HBV may have specific mechanisms to suppress cytokine production, highlighting the crucial role of these molecules in recovery from infection [175]. On the other hand, there are some of the immune evasion methods adopted by HBV include the antagonism of immune Function through the use of symmetrical of cytokine receptors, expression of viral proteins which react with cytokine signal transduction and expression of cytokine and host proteins that influence the Th1 and/or Th2 cytokine responses[172]. These immunomodulatory strategies can protect the host from the deadly inflammatory effects as well as inhibit the local inflammatory response

elicited to kill the HBV. The circulating cytokine profile in chronic hepatitis B was related to the replication level of the virus and the activity of liver disease [176]. In this respect, cytokine-based therapies could provide interesting approaches to achieve a functional cure or even the eradication of virus infection if the side effects can be managed [177].

However, both HBV and HCV often manage to escape the immune response. To this end, they interfere with various immune mechanisms including cytokine activity modulation [168].

1.12 Treatment and Vaccination of hepatitis B Virus:

The goal of therapeutics is to reduce a patient risk of death due to liver disease ideally to that of a person who has never been infected with HBV, or perhaps more realistically, to that of one with a resolved infection [76]. Currently, there are seven medications for the treatment of hepatitis B infection. These include antiviral drugs lamivudine (Epivir), adefovir (Hepsera), tenofovir (Viread), telbivudine (Tyzeka) and entecavir (Baraclude) and the two immune system modulators interferon alpha-2a and PEGylated interferon alpha-2a (Pegasys) [10].

HBV infection can be prevented by immunization, intramuscular administration of HBsAg leading to a protective anti-HBs response. The first vaccine (so-called plasma-derived vaccine) is produced by purifying HBsAg from the plasma of hepatitis B carriers. The second-generation vaccines contain HBsAg produced from yeast or (less commonly) mammalian cells by recombinant DNA technology [13]. The Hepatitis-B vaccine appears to be efficient in controlling HBV infection in children and adults. It was noticed that the HBsAb level is decreased by age, with increasing liability to get a disease, and that those with undetectable HBsAb also have a higher rate of infection [178]. Complete vaccination against hepatitis B is achieved by administration of a three-dose regimen,

with the second and third doses being given one and six months after the initial treatment. A test for anti-HBs should be carried out 6–8 weeks following the final dose of the primary course of vaccination [10]. Antibody levels of over 100 I.U/mL indicate an excellent response to vaccination. Antibody levels between 10 and 100 I.U/mL indicate an inadequate response and a booster dose should be given immediately to improve response. A blood test should be carried out 6–8 weeks after the booster dose to check answer [179].

All infants born to HBsAg-positive women should receive single-antigen hepatitis B vaccine and hepatitis B immune globulin (HBIG) (0.5 mL) <12 hours of birth, administered at different injection sites. The vaccine series should be completed according to a recommended schedule for infants born to infected mothers. The final dose in the vaccine series should not be administered before age 24 weeks (164 days) [180].

1.13 Aims of the Study:

Our study aims to detect the following markers:-

1. Serological markers (HBSAg, HBSAb, HBeAg, HBeAb, and HBCAb) were performed using Chromatographic immunoassay.
2. Quantitative detection of HBV DNA copies using real-time PCR in hepatitis B infection using commercially accredited kit utilizing TaqMan probe chemistry.
3. The comparison of plasma HBV DNA viral load in different states of hepatitis B infection.
4. Biochemical markers as an indication to predict of risk and progression of hepatitis B virus disease.
5. Oxidative stress markers (Malondialdehyde and superoxide dismutase and vitamin C) to investigate the changes in the oxidative stress in those patients and healthy control and studying the correlation between the Oxidative stress levels and another parameter.
6. Measuring the level of some cytokines including (IL-1α and IL-2) in patients and healthy control and investigating the correlation between the cytokines levels and another parameter.
7. Study the physiological effects of viral hepatitis B infections on different hematological parameters in patients and healthy subjects.
8. Estimate the association, if any, between the study parameters and viral hepatitis B infections to properly manage the treatment and disease progression.

Chapter Two

Materials and Methods

Chapter two

Materials and Methods

2.1-Materials:

2.1.1- Chemicals and instruments

The specific chemicals and instruments used in this study are listed in tables (2.1) and (2.2) respectively with its companies produced.

Table 2-1: Chemicals and their sources.

No	Chemicals & Their Sources	Company
1	Biochemical kits-liver function test	MNCHIP,China
2	Chronometric determination of Fibrinogen kit	Biolabo, France
3	ELISA kit for quantitative determination of Human Interleukin-1α	demeditec, Germany
4	ELISA kit for quantitative determination of Human Interleukin-2	demeditec, Germany
5	ELISA kit for quantitative determination of Human MDA level in serum	Shanghai, China
6	ELISA kit for quantitative determination of Human SOD level in serum	Shanghai, China
7	ELISA kit for quantitative determination of Human Vitamin C level in serum	Shanghai, China
8	HBV quantification kit: HBV Real-TM Quant: Real-Time kit for the quantitative detection of hepatitis B virus in human plasma	Sacace biotechnologies, Italy
9	Hematolog kits-complete blood count	SFRI, France
10	Prothrombin Time kit	Biolabo, France
11	Rapid immunochromatographic assay HBV 5 Rapid Test	CTK, Biotech
12	SaMag Viral Nucleic Acid Extraction Kit	Sacace –Italy
13	TOSO kit for detection of hepatitis B antibody in human serum	Human, Germany.

Table 2-2: Instruments and their manufacture company

N	Instruments	Company
1	Automated chemistry analyzer TOSOH, AIA-1800 ST	Japan
2	Celercare® M1 Automatic Chemistry Analyzer	MNCHIP, China
3	Centrifuge	Sigma, Germany
4	Centrifuge 3100 rpm for smart tubes	Cepheid, USA
5	Dry bath incubator	Major science, Taiwan
7	ELISA system (Reader, Washer, printer)	Awareness, USA
8	Eppendorf micropipettes with different sizes	Eppendorf, Germany
9	Freezer (-20 °C)	Denka, Korea
10	Genru: CA54	China
11	Hood (Fume cabinet)	Lab tech, USA
12	Incubator	Gallen Kamp, UK
13	Micropipettes 5-50, 0,5-10, 100-1000 µl	Eppendorf, Germany
14	Mini spin plus centrifuge	Eppendorf, Germany
15	Real-time PCR	Smart Cycler Cepheid, USA
16	Refrigerator	Samsung, Korea
17	SaMag-12/24 Automatic Nucleic Acids Extraction System	Sacace Biotechnologies, Italy
18	SFRI Blood cell counter	France
19	Vortex	Stuart SA7, England
20	Vortex shaker	Vortex- Genie, USA
21	Water Bath	Clifton, England
22	Water distillatory	Daihan, Turkey

2.2 Subjects:

A total of 80 patients infected with hepatitis B were included in this study. The duration of this study was from February to September 2018. After ethical clearance, the study was carried out in the General Al-Ameria Hospital/ Al-Anbar province. The study patients were divided into the following:

Group I: consists of 40 patients (20 males and 20 females) with the infective stage of hepatitis B (HBV) HBeAg positive, their age ranged between (3–67) years, with mean: (22.55 ±16.16) years.

Group II: consists of 40 patients (22males and 18 females) with Non-infectious phase of hepatitis B (HBV) HBeAg negative, their age ranged between (11-72) years, with mean: (31.45 ±13.08) years.

Forty healthy subjects were included as normal controls (healthy individuals) for the study (21 males and 19 females). Their age ranged between (3-60) years, with mean (29.57 ±16.30) years. These healthy volunteers with no clinical or laboratory evidence of liver disease. From all studied groups, a detailed history had been taken as shown in the Questionnaire (Appendix 1). A group with disease other than hepatitis B was excluded.

2.3 Specimens Collection:

About Ten ml of fresh blood were taken from each patient and healthy human by vein puncture. Serum was pooled from 6 ml of fresh blood in the sterile plastic tube and immediately stored at -20°C until use for serological investigations; biochemical tests including liver function tests, vitamins C, SOD, MDA, IL-1α and IL-2 were also included. Two ml were collected in sterile EDTA tube for complete blood count and then centrifuged within 3 hrs. at 2000 rpm for 20 minutes. Plasma was separated and transferred to sterile Eppendorf tubes and immediately stored at -20 °C will be used for molecular investigations. 1.8 ml from whole blood was adding to 0.2 ml (200 μl) 1.9 % trisodium citrate in the test tube and separated by centrifugation at 704 xg for 10 minutes to obtain plasma for Prothrombin Time test (PT) test, and fibrinogen test.

2.4 Methods:

2.4.1 Serological investigation:

2.4.1.1 Preliminary screening for HBsAg patients:

HBsAg level was determined according to the manufacturing instruction which laid down by fully automated chemistry analyzer TOSOH, AIA-1800

ST, Japan. The result is interpreted as appositive when the level is equal or more than 50 IU/ml and negative if the value is less than 50 IU/ml [13].

2.4.1.2 Hepatitis B profile:

Eighty plasma samples of patients and forty plasma samples of Control group or healthy subject was tested for the presence of HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb using HBV combo test device [13].

1. Principle:

Immunoassays for the detection of hepatitis B serological markers (Hepatitis B profile) have been done in plasma. The membrane is pre-coated with anti-HBsAg or anti-HBeAg antibodies on the test line region of the strip. During testing, the plasma specimen reacts with the particle coated with anti- HBsAg or anti-HBeAg antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg or anti-HBeAg antibodies on the membrane and generate a colored line. The presence of this colored line in the test line region indicates a positive result, while the absence of it indicates a negative result.

Hepatitis B surface antibody (HBsAb) is also known as anti-Hepatitis B Surface antigen (anti-HBs). This test is a qualitative, lateral flow immunoassay for the detection of HBsAb in plasma. The membrane is pre-coated with HBsAg on the test line region of the strip. During testing, the plasma specimen reacts with the particle coated with HBsAg. The mixture migrates upward on the membrane chromatographically by capillary action to react with HBsAg on the membrane and generate a colored line. The presence of this colored line in the test line region indicates a positive result. The disappearance of red color line indicates a negative result.

Hepatitis B envelope Antibody (HBeAb) is also known as anti-Hepatitis B envelope Antigen (anti-HBe). Hepatitis B core antibody (HBcAb) is also known as anti-Hepatitis B core antigen (anti-HBc). These tests are

immunoassays based on the principle of competitive binding. During testing, the mixture migrates upward on the membrane. Chromatographically by capillary action, the membrane is pre-coated with HBeAg or HBcAg on the test line region of the strip. During testing, the presence of anti- HBe antibody or anti-HBc antibody in the specimen will compete with particle coated anti-HBe antibody or anti-HBc antibody for a limited amount of HBeAg or HBcAg of the membrane, and no line will form in the test line region, indicating a positive result. A visible colored line will form in the test line region if there is no anti-HBe antibody or anti-HBc antibody in the specimen because of all the antibody. Coated particles will be captured by the antigen coated in the test line region. To act as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

2. Procedures:

1. The pouch was brought to the room temperature before opening it. The test device was removed from the sealed pouch.
2. The test device was placed on the clean and level surface. The dropper holds vertically, and three full drops of plasma (approx. 75 µl) transferred to each specimen well(s) of the test device respectively, and then the timer started.
3. I read the results at 15 minutes after, the red line (s) appeared.

3. Interpretation of the Results:

a. HBsAg, HBsAb, HBeAg:

Two red lines have appeared, one for control line region (C) and another line for test line region (T), indicating positive results of HBSAg, HBsAb, or HBeAg. The intensity of the red color in the test line region (T) was proportional to the concentration of HBsAg, HBsAb, and HBeAg in the specimen. The appearance of one red line for the control line region (C) and

no red or pink line for another test line region (T) indicated Negative results of HBSAg, HBsAb, HBeAg. Failure to appear for control line region (C) because of insufficient specimen volume or incorrect procedural techniques meant Invalidity.

b. HBeAb and HBcAb:

The appearance of one red line in the control line region (C) without the red or pink line in the test line region (T) indicated positive results for HBeAb and HBcAb. The appearance of two red lines in the control line region (C) and in the test line region indicated Negative results. Failure to appear for control line region (C) because of insufficient specimen volume or incorrect procedural techniques indicated Invalidity.

2.4.2 Molecular part of the study:

2.4.2.1 Automated Nucleic acids (HBV-DNA) extraction:-

SaMag Viral Nucleic Acids Extraction Kit)Sacace –Italy):

SaMag Viral Nucleic Acids Extraction Kit was designed to be used with SaMag-12 automatic DNA and RNA extraction system for the extraction of Viral DNA (HBV virus-DNA) from human biological specimens (plasma). The process of extraction consists of steps of lysis, binding, washing, and elution.

Specimen collection and conservation:

The purification procedure was optimized for use with 150 µl plasma. The frozen sample was thawed at room temperature (15–25°C), and the samples were processed immediately when they have equilibrated to room temperature. The supernatants were transferred to fresh tubes without disturbing the pellets, and the purification procedure had been started immediately.

Protocol

The extraction was performed starting with the SaMag-12 instrument, as follow:

Cartridges and reaction chambers were inserted. The tip holders, piercing pins, small tip, and filtered tips were inserted. Sample tubes in the sample rack were also entered, and 1.5 ml Elute tubes were embedded in the sample rack. Under a safe biological cabinet, samples were loaded in sample tubes. After that, the healthy internal subjects were added, and the sample rack was transferred into SaMag instrument. Then SaMag-12 door was closed, and the barcode was used to select Viral Nucleic Acids Extraction kit protocol HBV-DNA virus [(181)].

2.4.2.2 DNA concentration and purity:

The extracted genomic DNA from blood samples were checked by using Nanodrop Software (Bioneer /Korea). Moreover, the measurement of the purity of DNA through reading the absorbance in at (260 /280 nm) as the following steps:

1- The Nanodrop instrument was opened and selected the nucleic acid application, DNA.
2- An aliquot of (1 µl) of nuclease-free water was dispensed to lower optical surface lever arm.
3- Then, closed and the application software was selected for measuring on 260/280 nm wavelength.
4- The nucleic acid concentration was measured in the sample.
5- Nucleic acid concentration and purity ratios were automatically calculated by the software that was exhibited on the Personal Computer screen that is connected with Nanodrop [(182)]. If the outcome was 1.7-1.9, the sample was considered free of contamination and having a sufficient amount of DNA for further analysis.

2.4.2.3 qRT- PCR Quantification of HBV DNA Using Smart Cycler II:

HBV DNA extraction and real-time PCR amplification Qualitative and quantitative detection of plasma HBV DNA in all patients were performed

by Smart Cycler II real-time PCR (Cepheid, USA) using fluorescent reporter dye probes specific for HBV and HBV IC. Internal Control (IC) serves as an amplification control for each individually processed specimen and to identify possible inhibition. IC is detected in a channel other than the HBV DNA. Monitoring the fluorescence intensities during Real-Time allows the detection and quantification of the accumulating product without reopening the reaction tube after the amplification. The utilized kits for HBV DNA extraction and real-time PCR were done according to the procedures given in the kit protocols (Sacace Biotechnologies Italy). The sensitivity of the assay is 20 IU per ml blood sample. Specificity of the assay is about 99% [183].

Internal Control (IC):-

The internal Control HBV IC is quantitative Internal Control and represents recombinant DNA-containing-structure which carried through all steps of analysis from nucleic acid extraction to PCR amplification-detection. The presence of quantitative HBV IC allows not only to monitor the extraction procedure and to check possible PCR inhibition but also to verify possible losses of the DNA during extraction procedure and thus to enable the precise calculation of HBV viral load (according to Sacace company protocol).

2.4.2.3.1 Reagent Preparation for Amplification:

1. One set of reagents tubes thawed, vortex and centrifuged briefly. Reaction tubes or PCR plates were prepared.
2. Reaction Mix was prepared by adding of 200µl of PCR-mix-2-TM and 20µl of Hot Start DNA Polymerase into the tube with PCR-mix-1-TM. This mix was stable for one month at -20°C.
3. Vortex thoroughly and centrifuged briefly.
4. 12,5µl Reaction Mix was added into each tube.
5. 12,5µl of the extracted DNA sample was added to the appropriate tube

with Reaction Mix and mixed by pipetting.

6. Two Positive Controls and one negative control were prepared for each run:
   - Addition of 12,5µl Control pos.1 into the tube labeled Pos.1 and mixed by pipetting.
   - Addition of 12,5µl Control pos.2 into tube marked Pos.2. and mixed by pipetting.
   - Addition of 12,5µl Control neg. into the tube labeled Neg. Control.
7. Three HBV Standard were Prepared for each set of reagents as follows:
   - Addition of 12,5µl QS1 HBV into tube marked QS1 STD.
   - Acquisition of 12,5µl QS2 HBV into tube labeled QS2 STD.
   - Acquisition of 12,5µl QS3 HBV into tube labeled QS3 STD.
8. Three IC Standard were Prepared for each set of reagents as follow:
   - Addition of 12,5µl QS1 IC into tube marked IC1 STD.
   - Addition of 12,5µl QS2 IC into tube labeled IC2 STD.
   - Addition of 12,5µl QS3 IC into tube labeled IC3 STD.
9. The tubes were closed and transferred into the thermal cycler.

The PCR was then performed on a Smart Cycler II instrument (Cepheid) using primers and probes which were described previously. The PCR was run in a total volume of 25µl containing 12.5µl of the template, 12.5µl Reaction Mix. The amplification was performed as follows: An initial hot start denaturation at 95°C for 15min, and supported by 42 cycles of denaturation at 95°C for 20sec, annealing, and extension at 60°C for 40sec. Real-time monitoring was achieved by measuring the fluorescence at the end of the extension phase for each cycle. The quantitative analyses were conducted by using Smart Cycler II analysis software version 2.0. Following the manufacturer's instructions (Smart cycler: Cepheid, USA).

2.4.2.3.2 Program of Smart Cycler II Instrument:

The program used for amplification of HBV-DNA as follow 15 min

initial denaturation at 95 ^{o} C, followed by 42 cycles each of 20-second denaturation at 95 ^{o} C and 40-second annealing and extension at 60 ^{o} C as revealed in the following (Table 2-3).

Table 2-3 Program of RT- PCR for HBV Using Smart Cycler Instrument

Protocol Name		
Sacace HBV Real-TM Quant		
1- Stage 1	Hold	95 °C – 900 sec
2- Stage 2	Hold	95 °C – 20 sec
3- Stage 3	2- Temperature Cycle	60 °C – 40 sec Repeat – 42 times

The concentrations of the Quantitative Standards in the columns Fam Standard and Cy3 Standard to generate the Standard Curve (Table 2.4).

Table 2-4 Concentrations of the Quantitative Standards HBV and IC.

Quantitation standard HBV	Copies/Sample	Quantitation standard IC	Copies/Sample
QS1 HBV	**317600**	**QS1 IC**	**6810**
QS2 HBV	**2610**	**QS2 IC**	**7260**
QS3 HBV	**25**	**QS3 IC**	**690**

Fluorescence is observed in Real Time on the Cy3 channel for HBV DNA and FAM channel for Internal Control [185].

2.4.2.3.3 Results Analysis:

In the menu, under analysis setting, the value 10 was selected for both of channels Fam and CY3. Ct (Threshold cycle) on the Fam channel (IC) should be ≤ 30 for the clinical samples. If the Ct value of the specimen is higher than 30, a retesting of the sample is required.

2.4.2.3.4 Results Interpretation:

For each control and patient specimen, the concentration of HBV DNA was calculated using the following formula:

HBV DNA / IC DNA X coefficient* = copies HBV/ml [13].

(Copies/specimen) (Copies/specimen)

*coefficient is specific for each lot and reported in the HBV TM Quant Data Card provided in the kit. (Coefficient for this kit = 4.5 x 10 5).

2.4.3 Biochemical Investigations (Liver Function Test):-

The Celercare® M1 Chemistry Analyzer was used for detection of : Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total serum Bilirubin (TSB), Gamma-Glutamyltransferase (GGT) and Total protein (Albumin & Globulins) by using the liver function panel lyophilized kit (MNCHIP) [186].

Automatic Chemistry Analyzer:

1- The disc pouch was operated at the notch on the top right edge of the package, removed the reagent disc out and put on the table in a flat position.

2- One hundred µl volume pipettes were used to dispense approximately 100 µl of sample into the sample chamber via the sample port. After that, the sample chamber was filling.

3- The pipette tip was discarded into a biohazard container.

4- The reagent disc was cleaned. A lint-free tissue was used to eradicate sample spilled on the outlines of the disc.

5- After that, 500 µl volume pipette had been taken to dispense approximately 500 µl of sterile water for injection into the diluent chamber via the diluent port with the same procedure of ―Filling the sample chamber.

6- The analyzer was turned on by pressing the Power button on the front of the analyzer. After passing the self-test and reaching operating temperature.

7. Analyze was pressed and then the patient was selected, then the screen for choosing sample type was displayed:

8. Analyze was pressed and then the patient was selected, the screen for getting ready to scan QR code had been shown.

9. Before opening the disc foil pouch, the QR code was examined on the label of it to input the disc identification code, lot number, expiration date, and calibration data for the specific chemistries in the disc into the Analyzer, the screen for scanning QR code was displayed.

2.4.4 Oxidative Stress markers:-

2.4.4.1 Assay Procedure for Human Vitamin C (VC):-

The human vitamin C was purchased from (Shanghai, China) ELISA Kit with Cat.No： YHB3202Hu.

Principle

This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Vitamin C (VC). Add Vitamin C (VC) to wells that are pre-coated with Vitamin C (VC) monoclonal antibody and then incubate. After incubation, add anti-VC antibodies labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, and then add substrate A and B. The solution will turn blue and change to yellow with the effect of acid. The shades of the solution and the concentration of Human Vitamin C (VC) are positively correlated [(187)].

Assay procedure:

a) Dilution of standard solutions: (This kit provides one standard original concentration.

160ng/ml	Standard No.5	120µl Original Standard + 120µl Standard diluents
80ng/ml	Standard No.4	120µl Standard Number 5 + 120µl Standard diluents
40ng/ml	Standard No.3	120µl Standard Number 4 + 120µl Standard diluents
20ng/ml	Standard No.2	120µl Standard Number 3 + 120µl Standard diluents
10ng/ml	Standard No.1	120µl Standard Number 2 + 120µl Standard diluents

b) The number of stripes needed was determined by that of samples to be tested added by the standards.

c) Sample injection:

1- Blank well: The sample was not added, an anti-VC antibody labeled with biotin and streptavidin-HRP; chromogen reagent A & B were added, and the solution was stopped, each other step operation is the same.

2- Standard solution well: 50µl standard were added and streptomycin-HRP 50µl (biotin antibodies have united in advance in the standard, so no biotin antibodies are added).

3- The sample well to be tested: the 40µl sample was added and then 10µl VC antibodies, 50µl streptavidin-HRP. Then it was covered with a seal plate membrane. It was shaking gently to mix and incubated at 37°C for 60 minutes.

d) Preparation of washing solution: The washing concentration (30X) was diluted with distilled water for later use.

e) Washing: The seal plate membrane was carefully removed, the liquid was drained, and the remainder was shaken off. Each was filled well with washing solution, and let stand for 30 seconds, then was removed. This procedure was repeated five times then blots the plate.

f) Color development: Firstly, 50µl chromogen reagent A was added to each well, and then 50µl chromogen reagent B was added to each well. Gently shake to mix and incubated for 10 minutes at 37°C away from light for color development.

g) Stop: 50µl Stop Solution was added to each well to stop the reaction (color changes from blue to yellow immediately at that moment).

h) Assay: Blank was taken as well as zero; the absorbance (OD) of each well one was measured by one under 450 nm wavelengths, which should be conducted within10 minutes after having added stop solution.

i) According to standards concentrations and corresponding OD values, the linear regression equation of the standard curve was calculated. Then according to the OD value of the samples, the concentration of the

corresponding sample was calculated. The statistical software could also be employed.

Calculation

The concentration of standards was made in the abscissa and OD value the ordinate. The standard curve was drawn on the graph paper. According to the OD value of the sample, its similar concentration (which is the concentration of the sample) was located; or the linear regression equation of the standard curve was calculated according to the standard concentration and the OD value (Appendix 2). Then substituted with the OD value of the sample to estimate its concentration.

Assay range： 1ng/ml→300ng/ml.

Sensitivity: 0.52ng/ml.

2.4.4.2 Assay Procedure for Human Super Oxidase Dismutase (SOD):-

The human superoxide dismutase was purchased from (Shanghai, China)

ELISA Kit with Cat.No： YHB2870Hu.

Principle:

This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Super Oxidase Dismutase (SOD). Add Super Oxidase Dismutase (SOD) to wells that are pre-coated with Super Oxidase Dismutase (SOD) monoclonal antibody and then incubate. After incubation, add anti SOD antibodies labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, and then add substrate A and B. The solution will turn blue and change to yellow with the effect of acid. The shades of the solution and the concentration of human super oxidase dismutase (SOD) are positively correlated [188].

Assay procedure

a) Dilution of standard solutions: (This kit provides one standard original

concentration.

480U/L	Standard No.5	120μl Original Standard + 120μl Standard diluents
240U/L	Standard No.4	120μl Standard No.5 + 120μl Standard diluents
120U/L	Standard No.3	120μl Standard No.4 + 120μl Standard diluents
60U/L	Standard No.2	120μl Standard No.3 + 120μl Standard diluents
30U/L	Standard No.1	120μl Standard No.2 + 120μl Standard diluents

b) The number of stripes needed is determined by that of samples to be tested added by the standards. It is recommended that each standard solution and each blank well be arranged with multiple wells as much as possible.

c) Sample injection: 1) Blank well: Do not add sample, anti SOD antibody labeled with biotin and streptavidin-HRP; add chromogen reagent A & B and stop solution, each other step operation is the same. 2) Standard solution well: Add 50μl standard and streptomycin-HRP 50μl (biotin antibodies have united in advance in the standard, so no biotin antibodies are added). 3) The sample well to be tested: Add 40μl sample and then 10μl SOD antibodies, 50μl streptavidin-HRP. Then cover it with a seal plate membrane. Shake gently to mix. Incubate at 37°C for 60 minutes.

d) Preparation of washing solution: Dilute the washing concentration (30X) with distilled water for later use.

e) Washing: carefully remove the seal plate membrane, drain liquid and shake off the remainder. Fill each well with washing solution, let stand for 30 seconds, then drain. Repeat this procedure five times then blot the plate.

f) Color development: First add 50μl chromogen reagent A to each well, and then add 50μl chromogen reagent B to each well. Shake gently to mix. Incubate for 10 minutes at 37°C away from light for color development.

g) Stop: Add 50μl Stop Solution to each well to stop the reaction (color changes from blue to yellow immediately at that moment).

h) Assay: Take blank well as zero, measure the absorbance (OD) of each well one by one under 450 nm wavelength, which should be conducted within10 minutes after having added stop solution.

i) According to standards concentrations and corresponding OD values, calculate the linear regression equation of the standard curve. Then according to the OD value of samples, calculate the concentration of the corresponding sample. Statistical software could also be employed.

Calculation:

Make concentration of standards the abscissa and OD value the ordinate. the standard curve on the graph paper. According to the OD value of the sa locate its corresponding concentration (which is the concentration of the sam or calculate the linear regression equation of the standard curve according t standard concentration and the OD value (Appendix 3). Then substitute wit OD value of the sample to estimate its concentration.

Assay range： 3U/L→900U/L.

Sensitivity： 1.52U/L.

2.4.4.3 Assay Procedure for Human Malondialdehyde (MDA):-

The human malondialdehyde was purchased from (Shanghai, China)

ELISA Kit with Cat.No： YHB1955Hu.

Principle:

This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Malondialdehyde (MDA). Add Malondialdehyde (MDA) to wells that are pre-coated with Malondialchehyche (MDA) monoclonal antibody and then incubate. After incubation, add anti-MDA antibodies labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, and then add substrate A

and B. The solution will turn blue and change to yellow with the effect of acid. The shades of the solution and the concentration of Human [128].

Assay procedure:

a) Dilution of standard solutions: (This kit provides one standard original concentration.

32nmol/ml	Standard No.5	120µl Original Standard + 120µl Standard diluents
16nmol/ml	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
8nmol/ml	Standard No.3	120µl Standard No.4 + 120µl Standard diluents
4nmol/ml	Standard No.2	120µl Standard No.3 + 120µl Standard diluents
2nmol/ml	Standard No.1	120µl Standard No.2 + 120µl Standard diluents

b) The number of stripes needed is determined by that of samples to be tested added by the standards. It is recommended that each standard solution and each blank well be arranged with multiple wells as much as possible.

c) Sample injection: 1) Blank well: Do not add sample, anti-MDA antibody labeled with biotin and streptavidin-HRP; add chromogen reagent A & B and stop solution, each other step operation is the same. 2) Standard solution well: Add 50µl standard and streptomycin-HRP 50µl (biotin antibodies have united in advance in the standard, so no biotin antibodies are added). 3) The sample well to be tested: Add 40µl sample and then 10µl MDA antibodies, 50µl streptavidin-HRP. Then cover it with a seal plate membrane. Shake gently to mix. Incubate at 37°C for 60 minutes.

d) Preparation of washing solution: Dilute the washing concentration (30X) with distilled water for later use.

e) Washing: carefully remove the seal plate membrane, drain liquid and shake off the remainder. Fill each well with washing solution, let stand for 30 seconds, then drain. Repeat this procedure five times then blot the plate.

f) Color development: First add 50µl chromogen reagent A to each well, and

then add 50μl chromogen reagent B to each well. Shake gently to mix. Incubate for 10 minutes at 37℃ away from light for color development.

g) Stop: Add 50μl Stop Solution to each well to stop the reaction (color changes from blue to yellow immediately at that moment).

h) Assay: Take blank well as zero, measure the absorbance (OD) of each well one by one under 450 nm wavelength, which should be conducted within10 minutes after having added stop solution.

i) According to standards concentrations and corresponding OD values, calculate the linear regression equation of the standard curve. Then according to the OD value of samples, calculate the concentration of the corresponding sample. Statistical software could also be employed Malondialdehyde (MDA) are positively correlated.

Calculation:

Make concentration of standards the abscissa and OD value the ordinate. Draw the standard curve on the graph paper. According to the OD value of the sample, locate its corresponding concentration (which is the concentration of the sample); or calculate the linear regression equation of the standard curve according to the standard concentration and the OD value (Appendix 4). Then substitute with the OD value of the sample to estimate its concentration.

Assay range： 0.2nmol/ml→60nmol/ml.

Sensitivity： 0.14nmol/ml.

2.4.5 Cytokines Parameters:-

2.4.5.1 Assay Procedure for Human Interleukin-1α (IL-1α):

The human Interleukin-1α was purchased from (Demeditec, Germany) ELISA Kit with Cat.No： DE2432.

Principle:

An anti-human IL-1 alpha coating antibody was adsorbed onto microwells. Human IL-1 alpha present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human IL-1 alpha antibody was added and binds to human IL-1 alpha captured by the first antibody. Following incubation unbound biotin-conjugated anti-human IL-1 alpha antibody was removed during a wash step. Streptavidin-HRP was added and binds to the biotin-conjugated anti-human IL-1 alpha antibody. Following incubation, unbound Streptavidin-HRP was removed during a wash step, and substrate solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount of human IL-1 alpha present in the sample or standard. The reaction was terminated by addition of acid and absorbance was measured at 450 nm. A standard curve was prepared from 7 human IL-1 alpha standard dilutions and human IL-1 alpha sample concentration determined [189].

Assay procedure:

a. Determined the number of microwell strips required to test the desired number of samples plus an appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in a foil bag with the desiccant provided at 2°-8°C sealed tightly.

b. Washed the microwell strips twice with approximately 400 µl. Wash Buffer per well with the thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells. After the last wash step, empty wells and tap microwell strips on an absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively, Microwell

strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

c. Standard dilution on the microwell plate (Alternatively the standard dilution can be prepared in tubes): Added 100 µl of Sample Diluent in duplicate to all standard wells. Pipette 100 µl of prepared standard in duplicate into well A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 100 pg/ml), and transfer 100 µl to wells B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of human IL-1 alpha standard dilutions ranging from 100.0 to 1.6 pg /ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

d.100 µl of Sample Diluent in duplicate was added to the blank wells.

e. 50 µl of Sample Diluent was added to the sample wells.

f. 50 µl of each sample in duplicate was added to the sample wells.

g. Prepared Biotin-Conjugate.

h. Added 50 µl of Biotin-Conjugate to all wells.

i. Covered with an adhesive film and incubated at room temperature (18 to 25°C) for 2 hours, if available on a microplate shaker set at 400 rpm.

j. Prepared Streptavidin-HRP.

k. Removed adhesive film and empty wells. Washed microwell strips four times according to point b. Of the test protocol. Proceed immediately to the next step.

l. Added 100 µl of diluted Streptavidin-HRP to all wells, including the blank wells.

m. Covered with an adhesive film and incubated at room temperature (18° to 25°C) for 1 hour, if available on a microplate shaker set at 200 rpm.

n. Removed adhesive film and empty wells. Washed microwell strips four times according to point b. Of the test protocol. Proceed immediately to

the next step.

o. Pipette 100 µl of TMB Substrate Solution to all wells.

p. Incubated the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoided direct exposure to intense light. The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer appropriately recordable. Determination of the ideal period for color development has to be done individually for each assay.

It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively, color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9-0.95.

q. Stopped the enzyme reaction by quickly pipetting 100 µl of Stop Solution into each well. It was essential that the Stop Solution was spread rapidly and uniformly throughout the microwells to inactivate the enzyme completely. Results must be read immediately after the Stop Solution was added or within one hour if the microwell strips were stored at 2 - 8°C in the dark.

r. Read the absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength (optionally 620 nm as the reference wavelength; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Note: In the case of incubation without shaking the obtained O.D. values may be lower than Indicated below. Nevertheless, the results are still valid.

Calculation of results:

1. Calculated the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the

mean value.

2. Created a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human IL-1 alpha concentration on the abscissa. Draw the best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
3. To determine the concentration of circulating human IL-1 alpha for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve (Appendix 5). At the point of intersection, extend a vertical line to the abscissa and read the corresponding human IL-1alpha concentration.
4. If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µl sample + 50 µl Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).
5. Calculation of samples with a concentration exceeding standard one will result in incorrect, low human IL-1 alpha levels (Hook Effect). Such samples require further external predilution according to expected human IL-1 alpha values with Sample Diluent to precisely quantitate the actual human IL-1 alpha level.
6. It was suggested that each testing facility establishes a control sample of known human IL-1 alpha concentration and runs this additional control with each assay. If the values obtained were not within the expected range of the control, the assay results may be invalid.
7. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

2.4.5.2 Assay Procedure for Human Interleukin-2 (IL-2):

The human Interleukin-2 was purchased from (Demeditec, Germany) ELISA Kit with Cat.No：DE221

Principle:

An anti-human IL-2 coating antibody was adsorbed onto microwells. Human IL-2 presented in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human IL-2 antibody was added and binds to human IL-2 captured by the first antibody.

Following incubation unbound biotin-conjugated anti-human IL-2 antibody was removed during a wash step. Streptavidin-HRP was added and binds to the biotin-conjugated anti-human IL-2 antibody. Following incubation, unbound Streptavidin-HRP was removed during a wash step, and substrate solution reactive with HRP was added to the wells.

A colored product was formed in proportion to the amount of human IL-2 present in the sample or standard. The reaction was terminated by addition of acid and absorbance was measured at 450 nm. A standard curve was prepared from 7 human IL-2 standard dilutions, and human IL-2 sample Concentration determined [171].

Assay procedure:

a. 2 x 50 µl sample was needed for duplicate measurement. Serum or plasma samples, as well as reconstituted controls, were applied without predilution. Human IL-2 levels in cell culture supernatants may vary considerably. Optimal dilution has to be determined for each sample. For unknown cell culture samples, it was useful to analyze undiluted as well as prediluted samples (e.g., 1:20 - 1:50) in parallel, thereby covering a broader range in one assay. Cell culture supernatants with very high concentrations of human IL-2 require high dilutions (e.g., up to1:2000) to be measured correctly. Such samples must be prediluted in the respective cell culture medium.

b. Determined the number of microwell strips required to test the desired amount of samples plus an appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional

control sample should be assayed in duplicate. Remove extra microwell strips. From holder and store in a foil bag with the desiccant provided at 2°-8°C sealed tightly.

c. Biotin-Conjugate was prepared.

d. Washed the microwell strips twice with approximately 400 µl Wash Buffer per well with the thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells. After the last wash step, empty wells and tap microwell strips on an absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively, microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

e. Standard dilution on the microwell plate (Alternatively the standard dilution can be prepared in tubes): Added 100 µl of Sample Diluent in duplicate to all standard wells. Pipette 100 µl of adjusted standard (concentration = 2400.0 pg/ml) in copy into well A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 1200.0 pg/ml), and transfer 100 µl to wells B1 and B2, respectively .Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of human IL-2 standard dilutions ranging from 1200.0 to 18.8 pg/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

f. 100 µl of Sample Diluent in duplicate was added to the blank wells.

g. Added 50 µl of Sample Diluent to all sample wells.

h. 50 µl of each sample in duplicate was added to the sample wells.

i. Added 50 µl of Biotin-Conjugate to all wells.

j. Covered with an adhesive film and incubated at room temperature (18 to 25°C) for 2 hours, if available on a microplate shaker set at 400 rpm.

k. Streptavidin-HRP was prepared.

l. Removed adhesive film and empty wells. Washed microwell strips six times according to point d. of the test protocol. Proceed immediately to the next step.

m. 100 µl of diluted Streptavidin-HRP was added to all wells, including the blank wells.

n. Covered with an adhesive film and incubated at room temperature (18 to 25°C) for 1 hour, if available on a microplate shaker set at 400 rpm.

o. Removed adhesive film and empty wells. Washed microwell strips six times according to the point of the test protocol. Proceed immediately to the next step.

p. Pipette 100 µl of TMB Substrate Solution to all wells.

q. The microwell strips were incubated at room temperature (18° to 25°C) for about 30 min. Avoided direct exposure to intense light. The color development on the plate should be monitored, and the substrate reaction Stopped before positive wells are no longer appropriately recordable. Determination of the ideal period for color development has to be done individually for each assay. It was recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively, color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

r. Stopped the enzyme reaction by quickly pipetting 100 µl of Stop Solution into each well. It was essential that the Stop Solution was spread rapidly and uniformly throughout the microwells to inactivate the enzyme completely. Results must be read immediately after the Stop Solution was added or within one hour if the microwell strips were stored at 2 - 8°C in the dark.

s. The absorbance of each microwell on a spectrophotometer was read using

450 nm as the primary wavelength (optionally 620 nm as the reference wavelength; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Note: In the case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless, the results are still valid.

Calculation of results:

1. Calculated the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean value.
2. Created a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human IL-2 concentration on the abscissa. Draw the best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
3. To determine the concentration of circulating human IL-2 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve (Appendix 6). At the point of intersection, extend a vertical line to the abscissa and read the corresponding human IL-2 concentration.
4. Serum, plasma, and control samples have been diluted 1:2 (50 μl sample + 50 μl Sample Diluent (1x)) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).
5. If cell culture supernatants have been measured, the concentration read from the standard curve must be multiplied by the individual predilution factor (y) and dilution factor (2) of the assay (x 2y).
6. Calculation of samples with a concentration exceeding standard one may result in incorrect, low human IL-2 levels (Hook Effect). Such samples require further external predilution according to expected human IL-2 values with Sample Diluent to precisely quantitate the actual human IL-2 level.
7. It is suggested that each testing facility establishes a control sample of known human IL-2 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

8. A representative standard curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

2.4.6 Hematological tests:-

2.4.6.1 Complete Blood Count (CBC):

Complete blood counts (CBC) and differential were performed on the blood sample using Hematology analyzer (Blood Cell Counter), SFRI, France an automated 3-part differential hematology analyzer. The machine automatically dilutes whole-blood sample of 50 ml in the CBC/Differential mode, lyses and enumerates white blood cells (WBC), red blood cells (RBC), hemoglobin concentration (Hb), Packed Cells Volume (PCV), platelets, lymphocytes, neutrophils and the red cells indices (Mean Cell Volume, Mean Corpuscular Hemoglobin & Mean Corpuscular Hemoglobin Concentration) [(2)].

2.4.6.1.1 Sample: Fresh blood was drawn in anticoagulant tubes (contain EDTA or citrate).

2.4.6.2 Determination of Prothrombin Time (PT):

The BIOLAB SAS, Les Hautes Rives 02160 kit (Maizy, France) provide reagents for the determination of the prothrombin time (INR) in human plasma.

Principle:

The principle of the test consists of the clotting time is measured at 37 ^{0}C in the presence of tissular thromboplastin and calcium. The PT (in sec.) so measured is converted into PT (%) or INR [(99)].

Procedure:

The instructions of the instrument were followed, when measured PT, using Stago device, firstly calibration the equipment and add 50µl of plasma in cuvette then 50µl PT reagent were added and finally read directly in the device.

In case of testing by the manual method, first bring the Reagent 1 (i.e., the reconstituted thromboplastin reagent) to 37℃± 0.5; the steps were summarized as in the table below:

In a glass test tube or cuvette at 37 ℃ add:	
Plasma (standard, patients or control)	0.1 ml
Incubate at 37℃	2 min
Starting a stopwatch, add Reagent 1	0.2 ml
Mix. Note the clotting time (seconds)	

2.4.6.3 Determination of Fibrinogen:

The BIOLAB SAS, Les Hautes Rives 02160 kit (Maizy, France) provide reagents for the determination of the fibrinogen in human plasma.

Principle:

A method based on Von Clauss and al studies, validated by Destaing F and al. When an excess of thrombin is present, the pre-diluted plasma clotting time is in reverse order proportional to the fibrinogen's concentration in the specimen [99].

Procedure

Dilute plasmas 1/10 in dilution buffer. This dilution corresponds to a fibrinogen concentration in the test tubes between 200 to 400 mg/dl which is the optimal range for measurements.

In case of testing by the manual method, first, pre-incubate the thrombin in a water bath at 37℃, then dispense in test tubes as follow. Homogenise regularly the reagent during use:

In a glass test tube or cuvette at 37 ℃ add:	
Diluted Plasma (standard, patients or control)	0.2 ml
Incubate at 37℃	2 min
Add Thrombin	0.2 ml
Mix. Simultaneously start a timer and record the clotting time (seconds)	

2.5 Statistical Analysis:

The data were analyzed using Excel (Microsoft, Redmond, WA, USA), Minitab (ver. 17, Minitab, State College, PA, USA), and SPSS (ver. 24, IBM, Armonk, NY, USA). The results are expressed as means ± standard deviations. A chi-squared test for goodness of fit, Student's paired *t*-test, Student's independent *t*-test, and one-way analysis of variance analyses were used to examine statistical significance, as appropriate [(190)]. In addition, Pearson's correlation analysis was carried out to determine the relationships between all study variables in this work. Probability values < 0.05 were considered significantly different.

Chapter Three

Results and Discussion

Chapter Three

Results and Discussion

3.1 Demographic Presentation of Study Sample:

3.1.1 Gender:

Patients and controls were presented in terms of gender and age groups. For gender, hepatitis B envelope antigen positive patients (HBeAg +ve) were distributed as 20 males and 20 females with a ratio (1:1), while such frequencies among hepatitis B envelope antigen negative patients (HBeAg -ve) were 22 and 18, respectively with a ratio (1.2:1). In controls, males represented 21; while the female was 19 with a ratio (1.11:1) table (3-1). In this study, infected males were higher than females, but this difference was not significant by application of the chi-square test (p<0.05).

Table 3-1 Distribution of patients and controls according to gender.

Gender	(HBeAg positive) Patients (N=40)		(HBeAg negative) patients (N=40)		Healthy Control (N=40)		P- Value
	N	%	N	%	N	%	
Male	20	50.0	22	55.0	12	52.5	0.953 N.S
Female	20	50.0	18	45.0	19	47.5	0.949 N.S
P- Value	1.00 N.S		0.527 N.S		0.752 N.S		
M/F ratio	(1:1)		(1.2:1)		(1.11:1)		

3.1.2 Age:

The distribution of patients' infected and non-infected with hepatitis B virus and healthy control were divided into six age groups according to age (< 21, 21-30, 31-40, 41-50, 51-60 years and > 60 years). The age group < 21 years was more frequent in HBeAg positive patients (50.0%) and the age group > 60 years constitutes the least percentages of 5.0% in the first group. It was observed that the age range 21-30 year more

frequent in HBeAg negative patients (35%) and the age group > 60 years constitutes the least percentages, 2.5% in the second group as shown in (Table 3-2).

Table 3-2 Distribution of patients and controls according to age.

Age group (years)	(HBeAg positive) Patients (N=40)		(HBeAg negative) patients (N=40)		Healthy Control (N=40)	
	N	%	N	%	N	%
< 21	20	50.0	7	17.5	12	30.0
21 - 30	11	27.5	14	35.0	11	27.5
31-40	4	10.0	10	25.0	9	22.5
41-50	3	7.5	6	15.0	3	7.5
51-60	0	0.0	2	5.0	5	12.5
> 60	2	5.0	1	2.5	0	0.0
Mean±SD	22.55 ± 16.16		31.45 ± 13.08		29.57 ± 16.30	
Range	3 – 67		11-72		3 – 60	

These results have coincided partially with other studies carried out in Iraq such as Kadham who reported that the most common age group for hepatitis B (HBeAg positive, and HBeAg negative) was 20-29 years (third decade). The least age group affected in HBV patients were (> 61) which was the same as among HBeAg (+ve) patients and HBeAg (-ve) patients [(46)]. In a study done by Thageel, the researcher reported that most of HBV patients were located within 20-39 years group with a percentage of 47.7% and within the 40-59 years group with a percentage of 38.5%; whereas 8.5% and 5.3% were located within 60 years or more and less than 20 years groups, respectively [(191)]. The study of Al-Suraifi reported that patients ranged from 1-95 years with a mean of 40.5 years. Most of these patients were within the age group 41-50 years and 21-30 years with a percentage of 25.71% and 24.76%, respectively, followed by the age group 31-40 (20%), whereas the age group ≥80 years were constituted the least percentages (0.95%) [(192)]. Al-Kenzawi documented

that The hepatitis B virus infection was more frequent in the age group 31-40 (23.6%) with ratio 3:1 female to male (75 male and 25 female) followed by both the age group 21-30 and 41-50 which exhibit approximately the same frequency of infection (19.2%) and (18.4%) respectively. The age group 51-60 (17.6%); while the age group more than 60 years old (12%), and the age group which is less than twenty years old (9.2%) represents the less frequently age group infected with HBV [172]. Issa *et al*, revealed that in their study in Basra that the most infected age groups were 31-40 years with a percentage of 25.44% [193]. Al-Saffar distributed Hepatitis B patients into two age groups (<40 years and ≥ 40 years), the age group≥ 40 years are more frequent than the age group <40 years [168]. Al-Hamdani *et al*, reported that a high prevalence of HBV was in age groups <1year and ≥40 years [8].

Al-Qaysi was found that the more significant group of the infected persons with HBV were in the middle age (25-34 and 35-44) years old, while the lowest rate of infected persons was in the age group (1-14) years old [13]. Al-Hilli and Ghadhban reported that the most common age group for hepatitis B was the fourth decade [194]. Al-Waysi found that 45 year was the mean age for CHB patients [195], and Al-Azzawi who found that the most common age group affected was the third and fourth decade [196]. Several studies in the world also were agreed with this study as Sanchez-Tapias reported that the most common age group was the thirties [197]. Wasley *et al*, showed the higher prevalence of HBV marker was in age ≥50 years [198].

HBV infection in Iraq was contracted mostly in children and young adult, but perinatal transmission plays only a minor role in disease. The risk of hepatitis B infection may differ depending on gender, age, epidemiological information, and different cultural characteristics of the population [199]. An essential determinant of the epidemiology of HBV is

the age of acquisition of the virus. In areas with high endemicity, most infections occur in children (4-8 years), and an area with intermediate endemicity, the disease occurs in adolescents and adults, while in areas with low endemicity, most infections occur in adolescents and young adults (15-29 years) [(192)].

3.2 Virological Markers:

All patients were tested, using chromatographic immunoassays, to investigate the levels of HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb. The results for patients with infectious HBV (those who were positive for HBeAg) and those who were HBeAg-negative are shown in (Table 3-3). Similar testing showed that the healthy participants were negative for the presence of HBsAg, HBeAg, HBeAbs, and HBcAbs; 10 (25.0%) were positive for HBsAbs (due to previous vaccinations).

Table 3-3 Incidence of hepatitis B serological markers in patients positive and negative results for hepatitis B envelop antigens and in healthy control.

Marker	HBeAg positive patients (n = 40)		HBeAg negative patients (n = 40)		Healthy controls
	Positive no. (%)	Negative no. (%)	Positive no. (%)	Negative no. (%)	Negative no. (%)
HBsAg	40.0 (100)	0.0 (0.0)	40.0 (100)	0.0 (0.0)	0.0 (0.0)
Anti-HBsAg antibodies	0.0 (0.0)	40.0 (100)	1.0 (2.5)	39.0 (97.5)	10.0 (25)
Anti-HBeAg antibodies	1.0 (2.5)	39.0 (97.5)	29.0 (72.5)	11.0 (27.5)	0.0 (0.0)
Anti-HBc antibodies	27 (67.5)	13.0 (32.5)	39.0 (97.5)	1.0 (2.5)	0.0 (0.0)

3.2.1 Total Patients:

All study samples (from patients and controls) were investigated using both a serological method for detecting HBsAg levels and a molecular method for determining the HBV viral load. The first one is

the determination of Hepatitis B surface antigen (HBSAg level) by fully automated chemistry analyzer TOSOH, AIA-1880, and the second is the determination of HBV-DNA by (Real-Time PCR) for quantifying the viral load using fluorescent reporter dye probes (TaqMan probes) specific for HBV and HBV IC using the Smart Cycler II software protocol.

Out of the two virological markers (HBSAg level and HBV-DNA), the results showed high significant variations. Serum level of HBSAg was significantly increased in HBeAg positive patients compared to HBeAg negative patients (7779.9 ± 3898 *vs*. 3233.8 ± 2474 IU/mL). HBV-DNA level was also significantly increased in HBeAg positive patients compared to HBeAg negative patients (35328825 ± 23101537 vs. 3115.1± 1916.8 IU/mL), as shown in table (3-4).

Table 3-4 Hepatitis B virus surface antigen (HBsAg) levels and viral load (HBV-DNA copies) in hepatitis B virus envelope antigen-positive and -negative patients.

Virological Markers	Study Groups		P-Value
	HBeAg positive patients	HBeAg negative	
HBSAg level (IU/ml)	7779.9 ± 3898	3233.8 ± 2474	0.000**
HBV-DNA (IU/ml)	35328825 ± 23101537	3115.1 ± 1916.8	0.000**

Data presented as Mean ± SD, * significant differences (p<0.05), (**) highly significant differences (p<0.01).

Hepatitis B surface antigen is the universal marker used as a screen test in the diagnosis of HBV infection in suspected infected individuals. It was introduced over than twenty years ago as a qualitative marker for detecting HBV [200]. HBsAg is an important marker that not only indicates active HBV infection but can also predict clinical and treatment outcomes [10]. This antigen is considered as the key of HBV serological

markers in the diagnosis of the infection. It can be detected during three to four weeks after the first time of infection and might be reached to five months in acute infection. When it continues greater than six months, this indicates chronic hepatitis B infection [192]. The detection of HBsAg was considered to be a milestone in the history of HBV research and has been used for more than 40 years to confirm HBV infections. With the second generation of standard HBsAg samples, introduced by the World Health Organization, quantitative HBsAg tests have become possible [201]. Thus, in this study, we investigate the use of quantitative measurements of HBsAg levels in patient plasma samples (TOSOH, AIA-1880) as indicators of HBV infections. Our results clearly demonstrated that serum HBsAg levels were significantly increased in HBeAg-positive patients, compared with HBeAg-negative patients, in agreement with most other studies. However, Zhang et al, reported that on the role of immune status and concluded that serum HBsAg levels varied significantly among HBeAg-positive patients, depending on their immune status [202]. Their findings may have important implications for our understanding of HBV clearance in HBeAg-positive patients with CHB infections.

Occult HBV infections are recognized by the absence of circulating HBsAg in individuals positive for plasma or tissue HBV DNA, irrespective of other HBV serological markers [203]. These results are generally consistent with studies conducted in Iraq. Who reported on the distribution of HBV serological markers among patients with infectious (HBeAg-positive) and non-infectious (HBeAg-negative) forms of CHB infections [46]. Thus, the detection and quantification of HBV DNA plays an essential role in diagnosing and monitoring HBV infections as well as for assessing therapeutic responses [12]; our study provides another step in this direction.

In the present study, the viral load (HBV-DNA copies) is significantly higher in HBeAg-positive patients than in HBeAg-negative patients, in agreement with other published studies [46]. Mendy et al, [12] also quantified HBV-DNA copies in both HBeAg-positive and -negative patients and showed that 108 (34%) of 318 HBsAg-positive carriers had detectable HBeAg and all HBeAg-positive carriers tested positive for HBV-DNA compared with 164 (78%) of the HBeAg-negative carriers. The same researchers also showed that the geometric mean HBV-DNA concentration in HBeAg-positive carriers was higher than in HBeAg-negative carriers (8.7 log_{10} copies/mL vs. 4.1 log_{10} copies/mL, $p < 0.0001$). qRT-PCR measures the actual amount of HBV in a blood sample, helping to determine if the HBV is reproducing in the liver. In a person with a HBeAg-negative CHB infection, a viral load of >2000 IU/mL indicates the presence of a viral infection with the potential to cause liver damage [39]. Further, in a person with detectable HBeAg levels, a viral load of >20,000 IU/mL indicates that the virus is active and has the most significant potential to cause liver damage [204]. Thus, one of the conclusions of the present study is that qRT-PCR which is a sensitive, specific, and reproducible approach for detecting and quantifying HBV-DNA in clinical serum samples; it can also serve as an essential supplementary tool in clinical settings, especially for establishing viremia levels in HBeAg-positive or -negative patients with CHB infections.

In this study, quantitative measurement of HBV load in 80 HBV infected patients and 40 healthy controls is performed by Smart Cycler II real-time PCR (Cepheid, USA) using fluorescent reporter dye probes specific for HBV and HBV IC Internal Control (IC). A total of 80 HBsAg positive samples who screened by TOSOH analyzer, also

positive for qRT-PCR. Further 40 healthy controls showed negative result for HBsAg and qRT-PCR.

The standard curve was generated by using the Smart Cycler II software and serial dilution of known DNA concentration for HBV DNA and HBV Internal Control (IC) using fluorescent reporter dye probes specific for HBV and HBV IC, to quantitatively measure the number of viral DNA copy number/ml of the patients' blood. In high template concentration, amplification of specific DNA was observed during initial heating cycles. An increased fluorescence activity due to the cleaved probe leads to an increase in the reporter signal over time and the amount of reporter signal increase was proportional to the amount of product being produced for a given sample. The sequence detection instrument captured this increase and displayed by the software (Figure 3.1), (Figure 3.2).

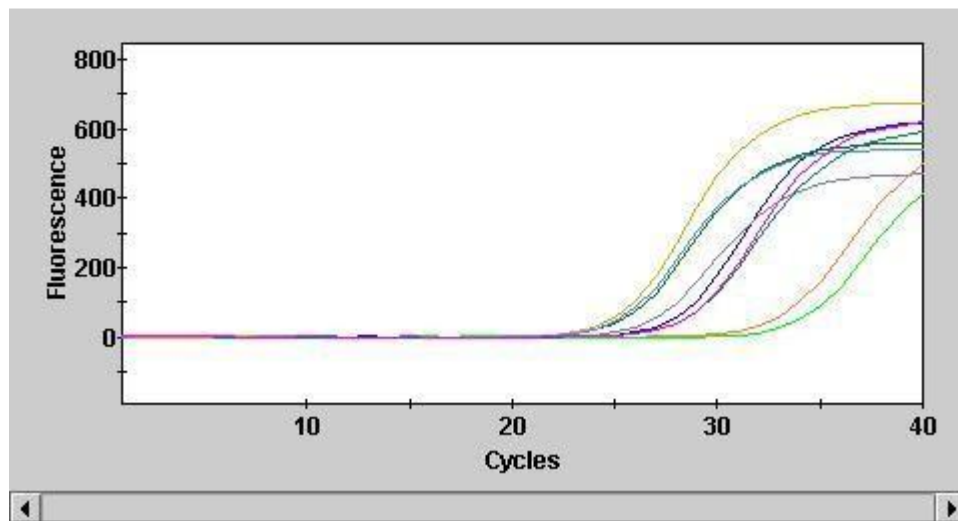


Figure 3.1 the amplification curve of HBV-DNA genome in RED Fluorescent channel detected by Real Time Polymerase Chain Reaction

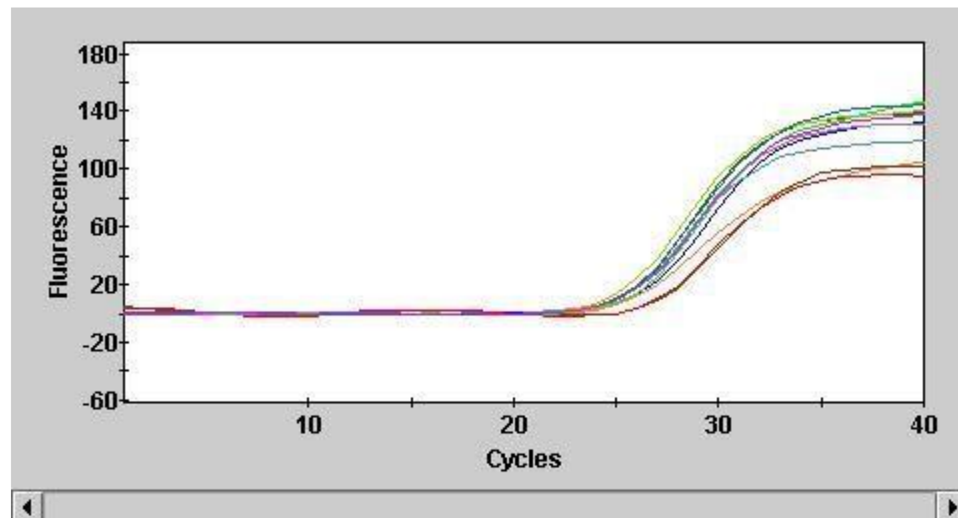


Figure 3.2 the amplification curve of HBV-DNA genome in FAM Fluorescent channel detected by Real Time Polymerase Chain Reaction

However, when subset analysis focused on such individuals, a relatively large proportion was found to have HBV-DNA levels > 2,000 copies/ml. This threshold was used since the effect of treatment in this population is unknown, making it impossible to distinguish those patients in whom low HBV-DNA levels are a result of therapy and seroconversion from those patients in whom the disease is inactive. By excluding those individuals with low viral load levels from this analysis[46].

In conclusion, serology will undoubtedly continue to be widely used in the diagnosis of HBV infection. However, significant advances have been made in the diagnosis and treatment of chronic HBV infection, and the HBV DNA amplification assays serve as valuable tools to monitor all these modalities. From this study, we conclude that real-time PCR is a sensitive, specific, and a reproducible approach for the detection and quantitation of HBV DNA in clinical serum samples. It can serve as an essential supplementary tool in a number of clinical settings, especially in detecting low levels of viremia in nonreplicative HBV disease and also in patients with past HBV infection. It is also useful for monitoring the efficacy of antiviral treatment. Further works

with detailed clinical and histological studies in the discordant cases are needed. [13].

3.2.2 Patients Distribution by Gender:

The patients were distributed into males and females revealed some significant differences in the serum level of HBSAg. Females HBSAg level showed a significant increase compared to male patients (HBeAg positive) (8799 ± 4184 *vs.* 6760± 3390 IU/mL). Male HBV patients (HBeAg negative) also showed a significant decrease of HBSAg level (2519± 1896 IU/mL) compared to female (4107 ± 2853 IU/mL). While HBV-DNA showed no significant difference results between males and females in each (HBeAg positive) and (HBeAg negative) patients as shown in table (3-5).

Table 3-5 Hepatitis B virus surface antigen (HBsAg) levels and viral load (HBV-DNA copies) in hepatitis B virus envelope antigen-positive and -negative patients distributed by gender.

Virological Markers	Study Groups			
	(HBeAg positive) patients		(HBeAg negative) patients	
	Male	Female	Male	Female
HBSAg level (IU/ml)	6760 ± 3390 b	8799 ± 4184 a	2519 ± 1896 b	4107 ± 2853 a
HBV-DNA (IU/ml)	29863029 ± 17308677 a	40794621 ± 27073337 a	3524.6 ± 2079 a	2614 ± 1614 a

Data presented as Mean ± SD, †Means that do not share a letter (Horizontally) within each group are significantly different (according to Independent T-test).

Both sexes can be affected by HBV. When the results distributed according to sex the data showed that females are more affected By HBsAg level than males. The difference is in the exposure rate to HBV infection between both sexes may be attributed to occupational factors and other risk factors associated with the exposure of females like physiological and hormonal differences between males and females. Our

results disagree with Khan *et al* [183] and Al-Qaysi [13] who concluded that the higher rates of HBV infections is observed in male more than in female. In our study, the results of T-test showed no significant difference between HBV-DNA results and sex. This is in agreement with what was observed by (Nita *et al.*) [205]. Also, this agrees with the study results done by (Al-Qaysi) [13].

3.2.3 Patients Distribution by Age Groups:

The distribution of patients into six age groups (< 21, 21-30, 31-40, 41-50, 51-60 years and > 60 years) revealed no significant differences in the serum level of HBSAg in each (HBeAg positive) and (HBeAg negative) patients, also HBV-DNA copies showed no significant differences result between different age groups in each (HBeAg positive) and(HBeAg negative) patients as shown in table (3-6), (Figure 3-3), (Figure 3-4).

Our results disagree with (Al-khozai) [206] who reported that most cases of HBsAg positive patients were the most significant ratio between the age of (21-51) years old, which is nearly similar to the results obtained by (Al-Qaysi) [13]. This may be attributed to that the sample size might account for such difference, and certainly, more diagnosed patients may give a better picture regarding the age and viral hepatitis.

Table 3-6 Hepatitis B virus surface antigen (HBsAg) levels and viral load (HBV-DNA copies) in hepatitis B virus envelope antigen-positive and -negative patients distributed by age groups.

Virological Markers	Age mean ± SD	(HBeAg positive patients)						(HBeAg negative patients)					
		< 21	21-30	31-40	41-50	51-60	> 61	< 21	21-30	31-40	41-50	51-60	> 60
HBsAg level IU/ml	Mean	7784 a®	6494 a	7511 a	11820 a	-	9285 a	4629 a	3132 a	3199 a	2227a	2892 a	1973 a
	SD	3769	3170	3198	7349	-	3415	2158	2767	2734	2017	1417	0
HBV-DNA IU/ml	Mean	42505352a	36097185 a	12372001 a	28912886 a	-	6375142 a	1318 a	3200 a	3632 a	3293 a	5000 a	4496 a
	SD	18979801	29609030	7916237	17493677	-	979638	439	1654	1764	2558	0	0

®Means that do not share a letter are significantly different horizontally within each group with (P<0.05), according to the least significant difference (LSD)

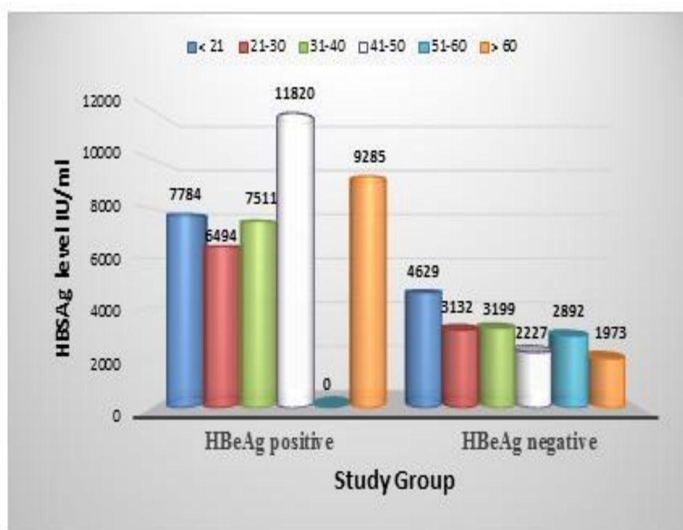


Figure 3-3 HBSAg level in the patient's groups

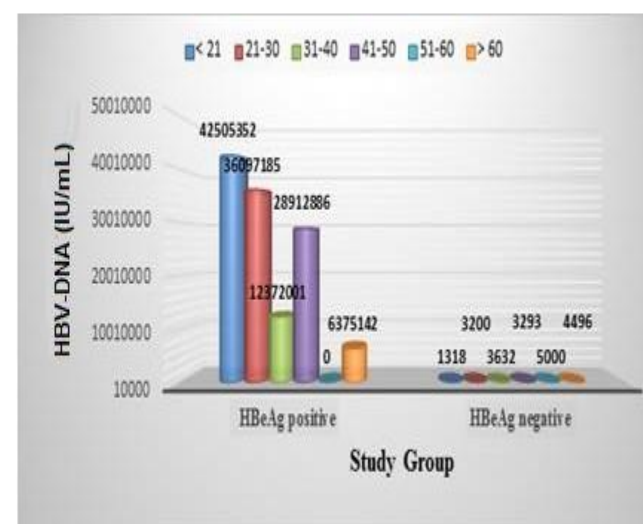


Figure 3-4 HBV-DNA in the patient's groups.

3.3 Biochemical Investigations (Liver function test):

3.3.1 Total Patients:

The statistical analysis (Table 3-7) shows the distribution of biochemical tests of (HBeAg positive) and (HBeAg negative) patients and healthy control, and the comparison between them. It was found that the level of AST among (HBeAg positive) patients group was higher than the (HBeAg negative) patients group and healthy control with the mean for the first was (34.01 ± 23.63 IU/L), (21.18 ± 10.3 IU/L) and (16.71± 3.5 IU/L) respectively and the difference was (P = 0.001). The level of ALT among (HBeAg positive) patients group was higher than the (HBeAg negative) patients group and healthy control with the mean for the first was (33.32 ± 24.55 IU/L), (22.24 ± 9.4 IU/L) and (18.39 ± 4.3 IU/L) respectively and the difference was (P = 0.001).

Regarding GGT levels it was found that it's mean level among (HBeAg positive) patients group (19.66± 12.20 IU/L) and (HBeAg negative) patients group (21.56± 10.4 IU/L) which were higher than the mean level of the healthy control (14.27 ± 3.5 IU/L) and the difference was (P = 0.002). ALP mean level was (98.86 ± 50.0 IU/L) in the first group which was higher than that of the second group and third group with the mean of (67.92 ± 36.9 IU/L), (58.02 ± 18.5 IU/L) respectively and the difference was (P = 0.001). The level of TSB, Direct Bilirubin, In Direct Bilirubin, TP, ALB, and Globulin showed no significant difference results in each (HBeAg positive) and (HBeAg negative) patients and healthy control.

Table 3-7 Serum biochemical investigations in both of two study groups (HBeAg positive and negative) Patients and healthy subjects

Chemical Parameters	(HBeAg positive)	(HBeAg negative) patients	Healthy Subjects	P-Value
AST (I.U/L)	34.01 ± 23.63 a	21.18 ± 10.3 b	16.71 ± 3.5 b	0.001**
ALT (I.U/L)	33.32 ± 24.55 a	22.24 ± 9.4 b	18.39 ± 4.3 b	0.001**
GGT (I.U/L)	19.66 ± 12.20 a	21.56 ± 10.4 a	14.27 ± 3.5 b	0.002*
ALP(I.U/L)	98.86 ± 50.0 a	67.92 ± 36.9 b	58.02 ± 18.5 b	0.001**
TSB (mg/dl)	0.77 ± 0.5 a	0.70 ± 0.1 a	0.69 ± 0.1 a	0.430 N.S
Direct Bilirubin (mg/dl)	0.192 ± 0.22 a	0.184 ± 0.07 a	0.191 ± 0.08 a	0.963 N.S
Indirect Bilirubin (mg/dl)	0.575 ± 0.25 a	0.515 ± 0.12 a	0.501 ± 0.13 a	0.141 N.S
Total protein (g/dl)	6.223 ± 0.54 a	6.200 ± 0.48 a	6.200 ± 0.48 a	0.973 N.S
Albumin (g/dl)	3.60 ± 0.26 a	3.71 ± 0.30 a	3.74 ± 0.31 a	0.080 N.S
Globulin (g/dl)	2.63 ± 0.33 a	2.49 ± 0.27	2.49 ± 0.29 a	0.071 N.S
Albumin/Globulin	1.38 ± 0.13 b	1.50 ± 0.16 a	1.51 ± 0.16 a	0.001**

Data presented as Mean ± SD, * significant differences (*p*<0.05), (**) highly significant differences (*p*<0.01). †Means that do not share a letter (Horizontally) are significantly different according to Least Significant differences Test (LSD).

ALT is found in kidney, heart, muscle and greater concentration in the liver compared with other tissues of the body. ALT is purely cytoplasmic catalyzing the transamination reaction AST catalyze transamination reaction. AST exist two different isoenzyme forms which are genetically distinct, the mitochondrial and cytoplasm form [(207)]. The increase of both aminotransferases enzymes strongly suggest hepatocellular injury, AST released from the damaged muscle tissues, red blood cells, and other location in addition to hepatocyte [(63)]. ALT, an enzyme released by hepatocytes during liver injury, usually reflects the degree of liver damage. ALT level is commonly used to assess the activity of the liver disease and

to identify patients who require treatment. However, ALT may be influenced by various factors, making it an imperfect surrogate marker [208].

In our study, the results of the AST, ALT level among (HBeAg positive) patients group is higher than the (HBeAg negative) patients group and healthy control. The result was in agreement with another study laid down by (Ahmed) [63]. He was demonstrated that the level of AST, ALT among HBeAg positive CHB patients group which was higher than the mean level of the HBeAg negative CHB patients group among Iraqi chronic hepatitis B patient's an inactive HBV carrier [63]. Increase in enzyme activity may be a result of the destruction of liver cells and the release of enzymes and an increase in their serum concentration. High levels of ALT are the most specific indicator of liver damage [69]. In hepatitis, ALT and AST become elevated with the progression of liver disease, likely as a result of direct hepatocellular damage and membrane leakage [209].

ALT and AST are used as the determinants for liver diseases, especially ALT which is the most reliable biochemical value to reveal the injury of hepatocytes. In the low level of damage to hepatocytes, the levels of ALT, which are dense in the liver cell cytosol, increases more than AST. If the injury of the hepatocytes is serious and there is necrosis, AST level is more than ALT [210]. AST elevations are often predominating in patients with cirrhosis and even in liver diseases that typically have an increased ALT [207].

Gamma-glutamyl transferase is the most sensitive biomarker of hepatobiliary disease. It is present in the cell membrane of nearly all human cells. GGT is most abundant in the kidney, liver, pancreas, and intestine, but the majority of GGT detected in serum derives from the liver [86]. In our study, the result of the GGT level was significantly increased in HBV (HBeAg positive), (HBeAg negative) patients. The

result was in agreement with another study laid down by (Bijedic et al) [189]. They demonstrated that the level of GGT in the hepatitis B patient's sera was also significantly increased in patients with chronic hepatitis compared to the levels in the control group [189]. High serum GGT levels may be considered an indicator of an advanced stage of fibrosis in patients diagnosed with chronic viral hepatitis B [88]. GGT has been known as one of the markers of oxidative stress because it is an enzyme playing an essential role in the extracellular catabolism of GSH as an indicator of intracellular antioxidant [211].

The higher levels of ALP activity may be involved in many parenchymal disorders of the liver, such as hepatitis [63]. In our study, the result of the ALP level is significantly increased in HBV patients. The result was in agreement with another study laid down by (Bijedic et al.) [189]. They demonstrated that the level of ALP in the hepatitis B patient's sera was also significantly increased in patients with chronic hepatitis compared to the levels in the control group [189]. ALP is the collective name given to several different enzymes that are capable of hydrolyzing phosphate esters at alkaline pH. These enzymes are widely distributed in the body, but the main sites of production are the liver, gastrointestinal tract, bone, placenta, and kidney. ALP in the liver is located in cell membranes of the hepatic sinusoids and the biliary canaliculi. Accordingly, levels rise with intrahepatic and extra hepatic biliary obstruction and with sinusoidal obstruction, as occurs in infiltrative liver disease [207]. Because these enzymes are found in liver cells (hepatocytes) which have lots of contact with blood supply, it can "leak" into the blood if the hepatocytes are damaged. Blood tests can determine the level of these enzymes in the blood, and doctors can use this information to form a diagnosis. Abnormally high levels of both liver enzymes show that liver cells have been damaged [29].

3.3.2 Patients Distribution by Gender:

The patients were distributed into males and females revealed some significant differences in the serum of total protein and globulin to (HBeAg positive) patients. Total protein level for females patients showed a significant increase compared to male patient in (HBeAg positive) (6.40 ± 0.58 *vs.* 6.05± 0.43 g/dl). Globulin level for females patients showed a significant increase compared to male patients in (HBeAg positive) (2.75 ± 0.34 *vs.* 2.49 ± 0.27 g/dl),while Male in (HBeAg negative) patients showed a significant increase of AST, ALT, GGT, TSB, and D.Bilirubin compared to females patients in (HBeAg negative) as shown in table (3-8).

Table 3-8 Serum biochemical investigations in both of two study groups (HBeAg positive and negative) Patients distributed by gender.

Chemical Parameters	(HBeAg positive) patients		(HBeAg negative) patients	
	Male	Female	Male	Female
AST (I.U/L)	27.60 ± 21.6 a	40.40 ± 27.4 a	24.64 ± 12.35 a	16.95 ± 4.39 b
ALT (I.U/L)	27.50 ± 19.30 a	39.20 ±28.2 a	26.07 ± 10.93 a	17.54 ± 3.78 b
GGT (I.U/L)	21.19 ± 15.63 a	25.25 ± 9.61 a	26.18 ± 11.75 a	15.91 ± 4.29 b
ALP(I.U/L)	102.8 ± 53.0 a	94.90 ± 47.8 a	64.16 ± 34.54 a	72.52 ± 40.06 a
TSB (mg/dl)	0.706 ± 0.10 a	0.83 ± 0.63 a	0.744 ± 0.17 a	0.64 ± 0.08 b
Direct Bilirubin (mg/dl)	0.146 ± 0.07 a	0.150 ± 0.04 a	0.206 ± 0.08 a	0.158 ± 0.04 b
Indirect Bilirubin (mg/dl)	0.55 ± 0.09 a	0.601 ± 0.34 a	0.54 ± 0.15 a	0.486 ± 0.08 a
Total protein (g/dl)	6.05 ± 0.43 b	6.40 ± 0.58 a	6.21 ± 0.47 a	6.189 ± 0.50 a
Albumin (g/dl)	3.55 ± 0.21 a	3.65 ± 0.30 a	3.77 ± 0.31 a	3.63 ± 0.28 a
Globulin (g/dl)	2.49 ± 0.27 b	2.75 ± 0.34 a	2.44 ± 0.25 a	2.55 ± 0.30 a
Albumin/Globulin	1.43 ± 0.12 a	1.33 ± 0.13 b	1.55 ± 0.16 a	1.43 ± 0.15 b

Data presented as Mean ± SD, †Means that do not share the same letter (Horizontally) within study groups are significantly different (according to independent T-test)

3.3.3 Patients Distribution by Age Groups:

The distribution patients into six age groups (< 21, 21-30, 31-40, 41-50, 51-60 years and > 60 years) revealed some significant differences between variables in (HBeAg positive) and (HBeAg negative) patients. In HBV (HBeAg positive) patients serum GGT It was found a higher level in the age ranged > 60 years with a mean of (44.8±26.87 IU/L), and the least level in the age ranged < 21 years with a mean of (15.05±5.74 IU/L). ALP it was found the higher level in the age ranged < 21 years with a mean of (129.65±36.36 IU/L), and the least level in the age ranged > 60 years with a mean of (46.60±7.78 IU/L).TSB it was found the higher level in the age ranged 41-50 year with a mean of (1.67±1.59 mg/dl), and the least level in the age ranged 31-40 year with a mean of (0.61±0.01 mg/dl). Direct Bilirubin was found the higher level in the age ranged 41-50 year with a mean of (0.62±0.76 mg/dl) and the least level in the age ranged > 60 years with a mean of (0.13±0.01 mg/dl). Indirect Bilirubin was found the higher level in the age ranged 41-50 year with a mean of (1.04±0.83 mg/dl) and the least level in the age ranged 31-40 year with a mean of (0.44±0.05 mg/dl). AST, ALT, TP, Albumin, and Globulin showed no significant differences between different age groups. In HBV (HBeAg negative) patients serum AST was found the higher level in the age ranged 51-60 year with a mean of (42.70±27.44 IU/L), and the least level in the age ranged 31-40 year with a mean of (14.92±2.19 IU/L). GGT was found the higher level in the age ranged 51-60 year with a mean of (39.80±14.28 IU/L), and the least level in the age ranged < 21 years with a mean of (15.83±3.04 IU/L). ALP was found the higher level in the age ranged < 21 years with a mean of (121.09±59.64 IU/L), and the least level in the age ranged > 60 years

with a mean of (44.20±0.00 IU/L). Direct Bilirubin was found the higher level in the age ranged 51-60 year with a mean of (0.33±0.18 mg/dl) and the least level in the age ranged < 21 years with a mean of (0.14±0.02 mg/dl) Table (3-9).

Table 3-9 Serum biochemical investigations in both of two study groups (HBeAg positive and negative) Patients distributed by age groups.

Chemical Parameters	Age mean ± SD	(HBeAg positive)patients						(HBeAg negative)patients					
		< 21	21-30	31-40	41-50	51-60	> 60	< 21	21-30	31-40	41-50	51-60	> 60
AST I.U/L	Mean	38.64 a	24.01 a	24.68 a	20.13 a	-	41.20 a	20.21 bc	20.00 bc	14.92 c	28.03ab	42.70 a	22.90abc
	SD	26.98	8.25	5.70	1.59	-	2.97	5.29	7.87	2.19	12.00	27.44	0.00
ALT I.U/L	Mean	37.09 a	23.25 a	23.68 a	30.37 a	-	30.75 a	22.81 a	21.77 a	17.42 a	26.27 a	36.00 a	21.10 a
	SD	26.82	5.86	7.06	2.83	-	0.07	4.30	10.20	4.23	12.09	20.08	0.00
GGT I.U/L	Mean	15.05 c	15.28 c	28.85 b	30.87ab	-	44.80 a	15.83 b	18.59 b	21.47 b	27.70 ab	39.80 a	30.80 ab
	SD	5.74	5.30	5.47	13.05	-	26.87	3.04	8.25	11.45	10.99	14.28	0.00
ALPI. U/L	Mean	129.65 a	56.36 c	68.65 bc	124.5 ab	-	46.60 c	121.09 a	57.05 b	55.03 b	58.98 b	61.05 b	44.20 b
	SD	36.36	13.32	7.66	100.95	-	7.78	59.64	16.45	17.67	10.59	23.55	0.00
TSB mg/dl	Mean	0.74 b	0.65 b	0.61 b	1.67 a	-	0.66 b	0.69 a	0.69 a	0.70 a	0.64 a	0.96 a	0.70 a
	SD	0.08	0.09	0.01	1.59	-	0.09	0.05	0.19	0.11	0.04	0.06	0.00
Direct Bilirubin mg/dl	Mean	0.15 b	0.17 b	0.17 b	0.62 a	-	0.13 b	0.14 b	0.18 b	0.18 b	0.20 ab	0.33 a	0.19 ab
	SD	0.04	0.09	0.04	0.76	-	0.01	0.02	0.05	0.08	0.06	0.18	0.00
Indirect Bilirubin mg/dl	Mean	0.59 b	0.48 b	0.44 b	1.04 a	-	0.53 b	0.55 a	0.51 a	0.52 a	0.44 a	0.64 a	0.51 a
	SD	0.08	0.09	0.05	0.83	-	0.10	0.06	0.18	0.07	0.08	0.12	0.00
Total protein g/dl	Mean	6.25 a	6.09 a	6.03 a	6.93 a	-	6.00 a	6.44 a	6.09 a	6.33 a	6.00 a	6.45 a	5.40 a
	SD	0.54	0.21	0.87	0.67	-	0.42	0.30	0.48	0.55	0.40	0.07	0.00
Albumin g/dl	Mean	3.62 a	3.59 a	3.48 a	3.83 a	-	3.35 a	3.80 a	3.59 a	3.82 a	3.65 a	3.99 a	3.40 a
	SD	0.26	0.19	0.28	0.40	-	0.21	0.12	0.33	0.35	0.26	0.16	0.00
Globulin g/dl	Mean	2.64 a	2.50 a	2.55 a	3.10 a	-	2.65 a	2.64 a	2.51 a	2.51 a	2.35 a	2.46 a	2.00 a
	SD	0.30	0.13	0.60	0.46	-	0.21	0.32	0.27	0.24	0.24	0.23	0.00
Albumin/Globulin	Mean	1.37 a	1.44 a	1.40 a	1.25 a	-	1.27 a	1.45 a	1.44 a	1.52 a	1.56 a	1.64 a	1.70 a

® Means that do not share a letter are significantly different horizontally within each group with (P<0.05), according to the least significant difference (LSD)

3.4 Oxidative Stress Parameters

3.4.1 Total patients

Table (3-10) shows the distribution of oxidative stress tests of HBV (HBeAg positive) and HBV (HBeAg negative) patients and healthy control, and the comparison between them. It was found that the level of V.C among (HBeAg positive) patients group was less than the (HBeAg negative) patients group and healthy control with the mean for the first was (1.79 ± 0.39 ng/ml) and (6.31 ± 2.0 ng/ml) and (6.29 ± 3.5 ng/ml) respectively and the difference was (P = 0.001). The level of SOD among (HBeAg positive) patients group and (HBeAg negative) patients group were less than healthy control with the mean for the first was (7.47 ± 1.1 U/L), (12.67 ± 3.7 U/L) and (95.06 ± 30.6 U/L) respectively and the difference was (P = 0.000).

Regarding MDA levels it was found that it's mean level among (HBeAg positive) patients group (2.19±1.3 nmol/ml) and (HBeAg negative) patients group (3.93± 2.7 nmol/ml) which were less than the mean level of the healthy control (16.99 ± 13.1 nmol/ml) and the difference was (P = 0.000).

Table 3-10 Serum levels of vitamin C, superoxide dismutase, and malondialdehyde healthy individuals and in patients with and without hepatitis B envelop antigen.

Oxidative Stress Parameters	HBeAg positive patients	HBeAg negative patients	Healthy Individuals	P-Value
V.C (ng/ml)	1.79 ± 0.39 b	6.31 ± 2.0 a	6.29 ± 3.5 a	0.001**
SOD (U/L)	7.47 ± 1.1 b	12.67 ± 3.7 b	95.06 ± 30.6 a	0.000**
MDA (n mol/ml)	2.19 ± 1.3 b	3.93 ± 2.7 b	16.99 ± 13.1 a	0.000**

Data presented as Mean ± SD, * significant differences (*p*<0.05),), (**)highly significant differences (*p*<0.01). †Means that do not share a letter (Horizontally) are significantly different according to Least Significant differencesTest (LSD).

In the present study, HBeAg-positive patients demonstrate the presence of significantly reduced serum vitamin C levels than were present in the HBeAg-negative patients and healthy volunteers. Significant reductions in serum vitamin levels, including vitamin C, are well-documented in patients with chronic viral hepatitis but the mechanisms through which these reductions are mediated have not been elucidated [(212)]. The same research has also documented that compounds exerting immune-stimulatory and antioxidant properties, like vitamin C, might be effective in reducing liver damage. Several vitamins have an essential role in neutralizing the activity of ROS and in regulating both innate and adaptive immune responses. Thus, patients with vitamin deficiencies, such as observed in the current study, are associated with increased susceptibilities to infections. This is especially true of HBeAg-positive patients with CHB infections [(213)].

Vitamin C plays a vital role in immune functioning and in different oxidative/inflammatory processes, such as reactive oxygen and nitrogen species scavenging, preventing the initiation of reactions that lead to protein glycation, and protecting against lipid peroxidation [(128)]. The oxidized products of vitamin C, ascorbyl radical and dehydroascorbic acid are easily regenerated to ascorbic acid by glutathione, NADH, or NADPH. Ascorbate can also recycle vitamin E and glutathione back from their oxidized forms [(144)]. For this reason, there has been interest in determining whether vitamin C can be used to combat the oxidative stress and subsequent inflammation associated with HBV infections.

In both HBeAg-positive and -negative patients, SOD levels were significantly lower than in healthy volunteers, as previously observed [(188), (214)]. Moulas et al. also observed significant reductions in serum erythrocyte SOD levels and in the total antioxidant status of patients chronically infected with HBV or HCV. Interestingly, these findings

were independent of the virological, biochemical, and clinical status of the patients. Hence, similar reductions were detected in patients with chronic but inactive HBV infections [214].

Enzymatic antioxidants, such as SOD, are among the first and most important lines of defense, and they act by scavenging potentially damaging free radicals. These types of antioxidants stop the release of hydroxyl radicals and prevent oxidative injury of cellular components. This is particularly important since free radicals are the primary agents causing vital organ (e.g., liver, kidney, and nervous system) toxicity. Because enzymes, like SOD, play essential roles in the protection of cells against oxygen toxicity [215], there has been interested in determining whether SOD levels are indicators of HBV-associated oxidative stress.

Similar to the SOD results, the MDA levels in the HBeAg-positive and -negative patients, in the current study, were significantly lower than in the healthy volunteers. However, these results are unlike those observed in other studies [188]. Previous studies have demonstrated that urine MDA levels and activity are increased in patients with HBV infections, compared with healthy study participants [216]. The reduced levels of MDA in our study may be explained by our hypothesis that when patients are immunocompetent, hydroxyl radicals (like MDA) are consumed (e.g., by hydroxylation), initiating free-radical chain reactions that subsequently decrease the MDA concentration. Nonetheless, MDA is the best indicator of lipid peroxidation and overall oxidative stress. MDA levels are particularly good indicators of oxidative stress in degenerative diseases, like hepatitis B. This is because, although the pathogenesis of chronic hepatitis is not fully known, HBV has been suggested to cause oxidative stress in infected cells. Further, lipid

peroxidation is one cause of hepatocyte damage and increased MDA levels in patients with hepatitis [216].

There is an abundance of evidence indicating that extensive oxidative stress occurs in patients with hepatitis B. This oxidative stress occurs on multiple levels, including lipid peroxidation, DNA oxidation, protein oxidation, and reactive oxygen and nitrogen species production. However, there are conflicting results concerning the value of antioxidant therapy in these patients, some of which may be explained by the concept of "compensatory gaps." Nevertheless, further studies are needed to better understand the potential benefits of antioxidant therapy in patients with HBV infections [102].

3.4.2 Patients Distribution by Gender:

The patients were distributed into males and females revealed no significant differences in the serum of V.C and SOD, and MDA in each (HBeAg positive) and (HBeAg negative) patients as shown in table (3-11).

Table 3-11 Serum levels of vitamin C, superoxide dismutase, and malondialdehyde in patients with and without hepatitis B envelop antigen distributed by gender.

Oxidative Stress Parameters	(HBeAg positive) patients		(HBeAg negative) patients	
	Male	Female	Male	Female
V.C (ng/ml)	1.61 ± 0.765 a	1.97 ± 1.09 a	6.17 ± 2.23 a	6.49 ± 1.64 a
SOD (U/L)	5.92 ± 4.73 a	9.04 ± 7.36 a	8.22 ± 7.21 a	14.80 ± 13.15 a
MAD (nmol/ml)	2.04 ± 1.29 a	2.34 ± 1.28 a	4.505 ± 2.95 a	3.219 ± 2.24 a

Data presented as Mean ± SD, †Means that do not share a letter (Horizontally) within the group are significantly different (according to independent t-test)

3.4.3 Patients Distribution by Age Groups:

Table (3-12) and (Figures 3-5, 3-6, and 3-7) shows the distribution of (HBeAg positive) and (HBeAg negative) patients and the comparison between them according to the age. Distribution patients into six age groups (< 21, 21-30, 31-40, 41-50, 51-60 years and > 60 years) revealed some significant differences between variables. In (HBeAg positive) patients serum V.C It was found higher level in the age ranged 31-40 year with a mean of (5.15 ± 3.35), and the least level in the age ranged > 60 years with a mean of (0.05 ± 0.04), while V.C in (HBeAg negative) patients showed no significant differences between different age groups. SOD and MDA showed no significant differences result between different age groups in each (HBeAg positive) and (HBeAg negative) patients. This may be attributed to that the sample size might account for such difference, and certainly, more diagnosed patients may give a better picture regarding the age and viral hepatitis.

Table 3-12 Serum levels of vitamin C, superoxide dismutase, and malondialdehyde in patients with and without hepatitis B envelop antigen distributed by age groups.

Oxidative Stress parameters	Age Mean ± SD	(HBeAg positive) patients						(HBeAg negative) patients					
		< 21	21-30	31-40	41-50	51-60	> 60	< 21	21-30	31-40	41-50	51-60	> 60
V.C (ng/ml)	Mean	1.05 bc	2.48 b	5.15 a	0.90 bc	-	0.05 c	4.12 a	6.87 a	7.15 a	8.68 a	0.67a	2.70 a
	SD	0.72	1.08	3.35	0.73	-	0.04	4	9.25	16.95	11.87	0.04	0
SOD (U/L)	Mean	7.97 a	9.38 a	6.01 a	3.37 a	-	1.18 a	11.19 a	14.18 a	13.91 a	13.68 a	4.11 a	11.80 a
	SD	4.93	8.73	5.04	1.71	-	0.11	10.25	9.28	7.21	11.66	2.31	0.00
MAD (nmol/ml)	Mean	1.67 a	2.72 a	3.01 a	3.36 a	-	1.06 a	2.54 a	3.87 a	3.64 a	4.2 a	7.57 a	8.41 a
	SD	0.92	0.96	0.95	3.00	-	0.58	0.7	2.78	2.82	2.84	2.72	0

Data presented as Mean ± SD, †Means that do not share a letter (Horizontally) within the group are significantly different according to independent t-test

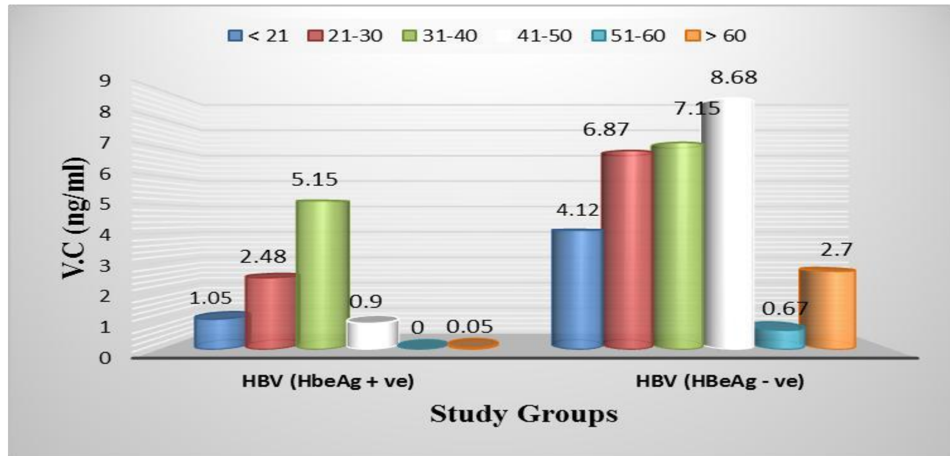


Figure 3-5 Vitamin C in the patient's groups.

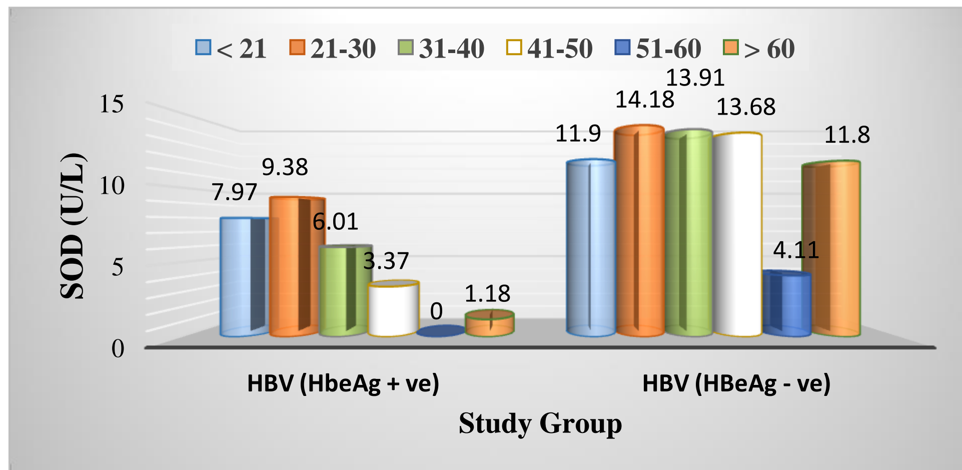


Figure 3-6 Superoxide dismutase in the patient's groups.

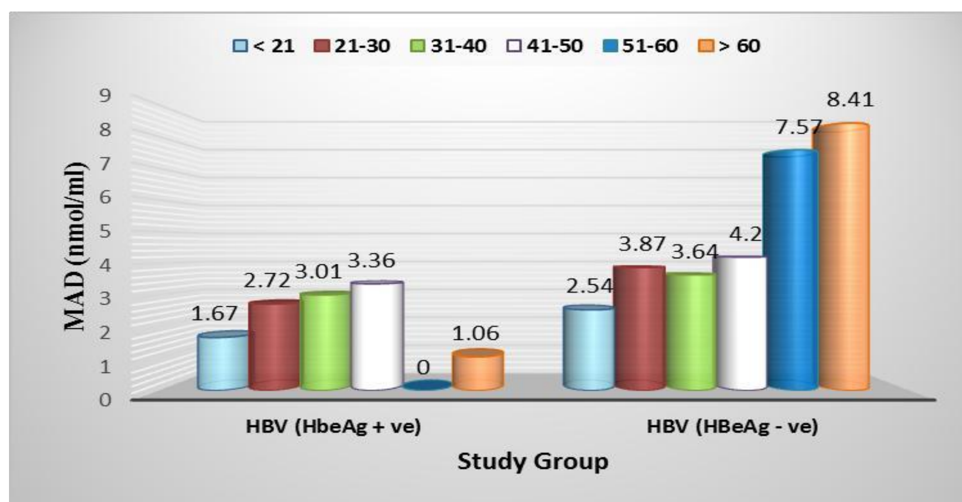


Figure 3-7 Malondialdehyde in the patient's groups.

3.5 Cytokines Parameters:

3.5.1 Total patients:

One of the two investigated cytokines (IL-1α and IL-2), IL-1α levels were similar in each of the 3 groups of individuals. IL-2 levels were not significantly different between the HBeAg-positive and healthy individuals, but the HBeAg-negative patients demonstrated significantly higher levels than either the HBeAg-positive patients or the healthy individuals (both, P = 0.001) (Table 3-13).

Table 3-13 Interleukin -1α and interleukin -2 levels in patients positive and negative for hepatitis B envelope antigens and in healthy individuals.

Cytokines Parameters	(HBeAg positive) patients	(HBeAg negative) Patients	Healthy Individuals	P-Value
IL-1α (pg/ml)	2.71 ± 1.1 a	3.12 ± 1.4 a	2.93 ± 0.8 a	0.255 N.S
IL-2 (pg/ml)	48.51 ± 29.3 b	78.42 ± 52.1 a	62.57 ± 11.6 b	0.001**

Data presented as Mean ± SD, * significant differences (p<0.05), (**) highly significant differences (p<0.01). †Means that do not share a letter (Horizontally) are significantly different according to Least Significant differenceTest (LSD).

HBV causes a hepatic inflammatory illness characterized by mononuclear and polymorphonuclear cellular infiltrates and evidence of hepatic macrophage activation [(171)]. These inflammatory cells produce several cytokines, including tumor necrosis factor, interferon, IL-1, and IL-6 [(149)], which mediate inflammatory processes, contribute to successful virus clearance, and help avoid infection progression and viral persistence [(171)]. IL-2 exerts a broad spectrum of effects on the immune system. In our study, IL-2 levels are significantly increased in HBV patients, in agreement with a study by Castillo et al. [(171)]. They demonstrated that IL-2 levels in the sera from patients with HBV infections (acute and convalescent phases) were significantly higher than

in the control group. IL-2 levels are well-documented to increase during the acute phase of HBV infections and that this increase is necessary to stimulate NK cell and CD8 lymphocyte activities and achieve remission [171]. Further, no significant IL-1α level differences were observed between patients with HBV and healthy volunteers. This result was unexpected as a prior study that showed significant increases in IL-1α levels in patients with CHB infections [189].

Lai et al. [165] suggested that immune-regulatory cytokines (i.e., IL-1 and IL-2) play essential roles in the host immune response to HBV infections and are critical for determining the outcome of HBV infections. When people are infected with HBV, immune cells secrete large amounts of cytokines that may result in viral clearance, persistent infection, or even liver damage [217]. Sustained increases in IL-2 levels during the convalescent phase suggest that, despite the resolution of the infection (as indicated by a return of liver function indicators to normal values and the presence of HBsAbs), increased hepatic damage would not be expected due to the high cytokine concentrations [171]. In addition, HBsAb mask tissue surface antigens, forming immune complexes that induce a transmembrane signal that suppresses the synthesis of intracellular viral antigens [218]. This, then, precludes the cytotoxic actions of CD8 lymphocytes.

Immediately following viral infection, a strong host response is initiated that includes activation of pre-existing anti-viral defense mechanisms that contribute to cellular apoptosis and the production of specific cytokines. These events lead to reduced viral replication and limit the spread of the virus [219].

3.5.2 Patients Distribution by Gender:

The patients were distributed into males and females revealed some significant differences in the serum of IL-2 to (HBeAg negative) patients. IL-2 level in females with (HBeAg negative) patients showed a significant decrease compared to male with (HBeAg negative) patients (57.00± 40.59 *vs.* 95.9± 54.7 pg/ml), but male and female with (HBeAg positive) patients showed no significant difference between them, while IL-1α showed no significant difference results between males and females in each (HBeAg positive) and (HBeAg negative) patients as shown in table (3-14).

Table 3-14 Interleukin -1α and interleukin -2 levels in patients positive and negative for hepatitis B envelope antigens distributed by gender

Cytokines Parameters	(HBeAg positive) patients		(HBeAg negative) patients	
	Male	Female	Male	Female
IL-1α (pg/ml)	2.79 ± 0.89 a	2.64 ± 1.22 a	3.00 ± 1.47 a	3.28 ± 1.29 a
IL-2 (pg/ml)	47.17 ± 24.45 a	49.86 ± 34.08 a	95.9 ± 54.7 a	57.00 ± 40.59 b

Data presented as Mean ± SD, †Means that do not share a letter (Horizontally) within a group are significantly different (according to independent t-test)

The results of cytokine serum levels distributed according to gender demonstrated that Il-2 revealed significant variations among the investigated groups, while no significant difference was observed in the level of IL-1α. Our results disagree with AL-Saffar as they find no significant difference between male and female for serum IL-2 in HBV patients [(168)]. Previous studies have demonstrated a correlation between cytokine serum levels and gender, and this may have been influenced by various factors, and among them is the hormonal status [(220)]. In this regard, immune defense capacity has shown differences between human males and females. In addition, males are found to be more prone to

infections, while females are at higher risk to develop autoimmune diseases [221]. These findings were correlated with humoral responses to the foreign antigenic challenge, and the suggestion was that sex hormones might influence immune functions [222]. Concerning cytokines, Elahe *et al,* reported that inflammatory cytokines were differentially regulated in response to hepatic infection in male and female mice [223]. In addition, Klingstrom *et al,* revealed that the cytokine responses in females and males are not similar during acute viral infection; implying that the subsequent activation and function of immune responses might differ between female and male patients upon infection [224]. These findings may explain part of the observed sex differences in susceptibility to infectious diseases and mortality following viral infections.

3.5.3 Patients Distribution by Age Groups:

The distribution of patients into six age groups (< 21, 21-30, 31-40, 41-50, 51-60 years and > 60 years) revealed no significant differences in the serum level of IL-1α in each (HBeAg positive) and (HBeAg negative) patients, also IL-2 showed no considerable differences result between different age groups in each (HBeAg positive) and (HBeAg negative) patients as shown in table (3-15), (Figure 3-8), (Figure 3-9).

Table 3-15 Interleukin -1α and Interleukin -2 levels in patients positive and negative for hepatitis B envelope antigens distributed by age groups.

Cytokines Parameters	Mean ± SD	HBV (HBe + ve)						HBV (HBe - ve)					
		< 21	21-30	31-40	41-50	51-60	> 60	< 21	21-30	31-40	41-50	51-60	> 60
IL1alpha pg/ml	Mean	2.61 a	2.46 a	3.46 a	3.26 a	-	2.85 a	3.24 a	3.57a	2.85 a	2.86 a	2.46 a	1.92 a
	SD	0.96	0.87	2.03	0.65	-	1.21	1.36	1.80	1.12	0.80	0.80	0.00
IL2 pg/ml	Mean	39 a	55 a	47 a	68 a	-	78 a	42 a	70 a	99 a	81 a	131 a	105 a
	SD	14.97	39.61	34.57	41.37	-	36.42	18.31	54.37	54.34	59.85	2.12	0.00

Data presented as Mean ± SD, †Means that do not share a letter (Horizontally) within the group are significantly different according to independent t-test.

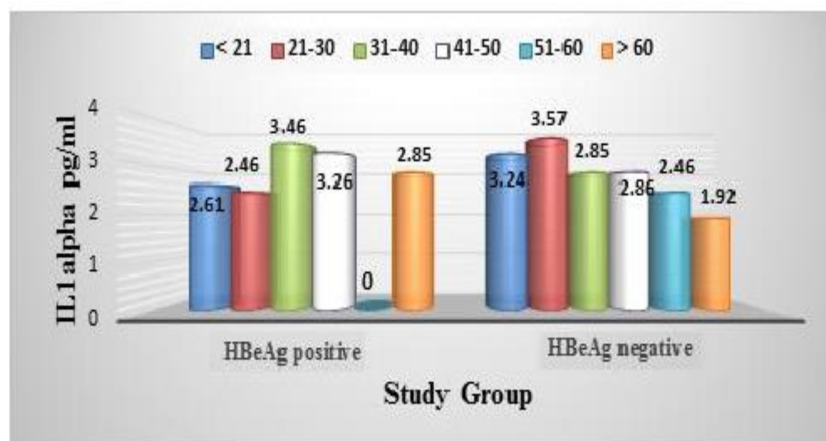


Figure 3-8 Interleukin -1 α in the patient's groups.

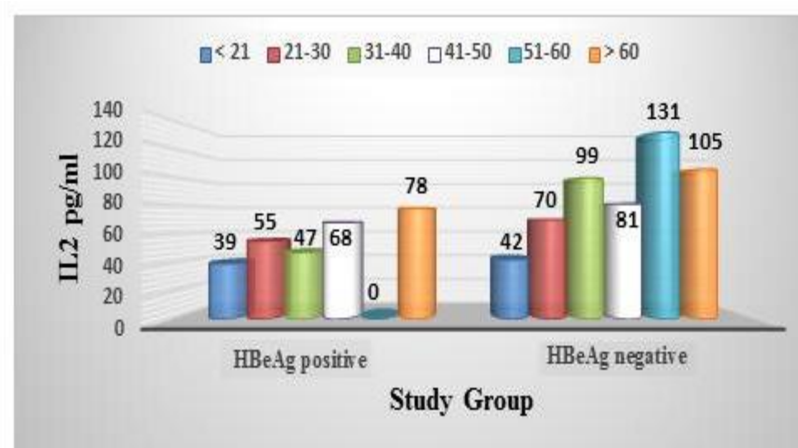


Figure 3-9 Interleukin -2 in the patient's groups.

Such results may suggest that the age is not critical factor that affects the serum level of the two investigated cytokines; however, caution must be considered in interpreting these results because the sample size in patients and controls may not permit a firm conclusion. Also, age can be regarded as an affected factor when we have subjects at age more than 60 years because it is often that a dysregulation in the immune functions and a decline in health and increased sensitivity to various diseases are associated with advanced ages [(225)]. In addition to that, cytokines are central to immune cell communications, age-associated changes in cytokine production may contribute to these alterations [(225)].

Unfortunately, the number of patients and controls of the present study at ages more significant than 60 years could not permit a careful statistical analysis, and therefore they were distributed into six age groups (< 21, 21-30, 31-40, 41-50, 51-60 years and > 61 years). However, in agreement with the present findings, AL-Saffar found that IL-2, IL-4, IL-10 showed no significant differences between both age groups (< 40 and ≥ 40 years) in HBV patients [(168)].

3.6 Hematological Parameters:

3.6.1 Total patients:

The statistical analysis (Table 3-16) shows the distribution of hematological tests of HBV (HBeAg positive) and HBV (HBeAg negative) patients and healthy control, and the comparison between them. It was found that the level of Prothrombin time among (HBeAg positive) patients and (HBeAg negative) patients groups were higher than the healthy control group with the mean for the first was (15.24 ± 2.0 sec), (15.73 ± 2.4 sec) and (13.65 ± 0.5sec) respectively and the difference was (P = 0.001) and the level of INR among (HBeAg

positive) patients group and (HBeAg negative) patients group were higher than the healthy control group with the mean for the first was (1.31 ± 0.3), (1.4o ± 0.4) and (1.06 ± 0.1) respectively and the difference was (P = 0.001). Regarding Monocytes % it was found that it's mean level among (HBeAg positive) Patients group (8.92 ± 2.5 %), and (HBeAg negative) patients group (7.81 ± 2.9 %) which were less than the mean level of the healthy control (11.18 ± 2.9 %) and the difference was (P = 0.000). Granulocytes % mean level was (62.12± 9.4I %) in second group (HBeAg negative) patients who were higher than that of the third group healthy control and with the mean of (55.31± 9.4 %), and the difference was (P = 0.015). RBC mean level was (4.97± 0.5) in second group (HBeAg negative) patients who were higher than that of the third group healthy control and with the mean of (4.59 ± 0.4), and the difference was (P = 0.002).

HCT (PCV) % was found that it's mean level among (HBeAg positive) patients group (41.49 ± 5.0 %) and (HBeAg negative) patients group (43.24 ± 5.0 %) which were higher than the mean level of the healthy control (39.12 ± 3.7 %) and the difference was (P = 0.001). MCHC it was found that it's mean level among (HBeAg positive) patients group (31.54 ± 1.10 g/dl) and (HBeAg negative) patients group (31.33 ± 1.37 g/dl) which were less than the mean level of the healthy control (33.02 ± 0.74 g/dl) and the difference was (P = 0.000). And the level of Platelets among (HBeAg positive) patients group was higher than (HBeAg negative) patients group, and healthy control group with the mean for the first was (302.63 ± 91.8), (236.8 ± 72.3) and (258.4 ± 81.8) respectively and the difference was (P = 0.002), while the level of Fibrinogen, WBC, Lymphocyte %, HB, MCV, and MCH showed no significant difference results in each HBV (HBeAg positive) and HBV(HBeAg negative) patients and healthy control.

Table 3-16 Hematological investigations in both of two study groups (HBeAg positive and negative) Patients and healthy subjects

Hematological Parameters	(HBeAg positive) patients	(HBeAg negative) Patients	Healthy Subjects	P-Value
Fibrinogen (mg/dl)	276.39 ± 75.4 a	263.47 ± 81.3 a	242.76 ± 26.0 a	0.074 N.S
Prothrombin time (sec.)	15.24 ± 2.0 a	15.73 ± 2.4 a	13.65 ± 0.5 b	0.001**
INR	1.31 ± 0.3 a	1.40 ± 0.4 a	1.06 ± 0.1 b	0.001**
WBC *10^3	8.33 ± 2.5 a	7.42 ± 1.9 a	7.49 ± 1.3 a	0.076 N.S
Lymphocyte %	32.81 ± 10.8 a	30.07 ± 8.2 a	33.51 ± 8.0 a	0.207 N.S
Monocytes %	8.92 ± 2.5 b	7.81 ± 2.9 b	11.18 ± 2.9 a	0.000**
Granulocytes %	58.20 ± 12.0 ab	62.12 ± 9.4 a	55.31 ± 9.4 b	0.015*
RBC *10^6	4.79 ± 0.5 ba	4.97 ± 0.5 a	4.59 ± 0.4 b	0.002**
Hemoglobin (g/dl)	13.08 ± 1.6 a	13.60 ± 1.9 a	12.92 ± 1.3 a	0.154 N.S
HCT (PCV) %	41.49 ± 5.0 a	43.24 ± 5.0 a	39.12 ± 3.7 b	0.001**
MCV (fl)	86.60 ± 6.2 a	87.24 ± 7.1 a	85.48 ± 5.6 a	0.454 N.S
MCH (pg)	27.33 ± 2.18 a	27.36 ± 2.82 a	28.23 ± 1.97 a	0.154 N.S
MCHC (g/dl)	31.54 ± 1.10 b	31.33 ± 1.37 b	33.02 ± 0.74 a	0.000 **
Platelets *10^3	302.63 ± 91.8 a	236.8 ± 72.3 b	258.4 ± 81.8 b	0.002**

Data presented as Mean ± SD, * significant differences (*p*<0.05), (**) highly significant differences (*p*<0.01). †Means that do not share a letter (Horizontally) are significantly different according to Least Significant differenceTest (LSD).

Hematological parameters provide information regarding the status of bone marrow activity and hemolysis [226]. The liver plays a vital role in hemostasis as most of the coagulation factors, anticoagulant proteins, and components of the fibrinolytic system are synthesized by the hepatic parenchymal cells [99].

This study has demonstrated the effect of HBV on some hematological parameters among hepatitis B envelop antigen-positive negative chronic hepatitis B patients and controls. Fibrinogen is a glycopeptide that facilitates the formation of blood clots. It is synthesized in hepatocytes and plays a central role in hemostasis [227]. In our study, the result of the fibrinogen level has no significant difference

results in each (HBeAg positive) and (HBeAg negative) patients and healthy control. The result was not compatible with another study led by (Leticia et al.) [99].They demonstrated that the level of fibrinogen in the hepatitis B patient's sera was significantly increased compared to the levels in the control group [99]. The extent of coagulation abnormalities depends on the severity of liver dysfunction, and the clinical manifestations of the liver disease depend on the severity of hemostatic impairment [97].

The prothrombin time (PT), is a clot-based test of the extrinsic and common coagulation pathways [228]. In our study, the result of the PT level was significantly increased in HBV (HBeAg positive), (HBeAg negative) patients. The result was in agreement with another study led by (Leticia et al.) [99].They demonstrated that the level of PT in the hepatitis B patient's sera was significantly increased compared to the levels in the control group [99]. These changes can, therefore, be explained based on the state of the diseased liver which is saddled with the responsibility of clotting factors synthesis [229].

International normalized ratio (INR) or standardized prothrombin time, as the name suggest needs a detailed clarification of PT (prothrombin time) [230]. In our study, the result of the INR level is significantly increased in HBV (HBeAg positive), (HBeAg negative) patients. The result was in agreement with another study led by (Balkan et al.) [231].They demonstrated that the level of INR was significantly lower in inactive carriers compared with CHB patients [231]. The best application of INR to a patient with liver disease is to monitor the degree of impairment of synthetic function or to predict mortality. In patients with abnormal coagulation testing results in the setting of liver disease, INR and PT may be best used to provide the practitioner with

information about the synthetic function of the liver but not to assess hemorrhagic risk [232].

WBC is usually raised due to infectious disease (viral and bacterial infection) and cause inflammation in infected via infiltrating of inflammatory cells to the site of inflammation [233]. In our study, the result of the WBC level has no significant difference results in each of HBeAg positive and negative patients and healthy control. The result was not compatible with another study led by (Ifeanyi et al.) [234].They demonstrated that the result equally showed a significant difference ($P<0.05$) in WBC of HBV subjects compared to the controls [234]. Abnormalities in hematological allusion into so much come across in cirrhosis. Several reasons contribute to the incidence of hematological abnormalities according to new studies that the existence of hematological cytopenias is linked to poor prognosis in cirrhosis [235].

Clinical experience suggests that platelet parameters may be useful in the assessment of liver inflammation and fibrosis, and platelet parameters are commonly evaluated during routine blood tests [236]. In our study, the result of the PLT level is increased significantly in (HBeAg positive) patients compared with (HBeAg negative) patients and healthy control. The result was not compatible with another study led by (ALKINANI) [237], which demonstrated that no significant difference between HBV patients and control in the level of PLT [237]. Although the results of PLT in the current study is upper in (HBeAg positive) patients than those in healthy group and(HBeAg negative) patients , statistical analysis revealed that there was no significant difference between (HBeAg negative) patients and healthy group, and this may be due to the difference in virulence strain of the virus [237].

HBV virus could affect bone marrow megakaryocytes and cause thrombocytopenia. In addition, liver fibrosis affects the production of

thrombopoietin, but studies have found no significant relationships between platelet count and thrombopoietin concentrations [(236)]. Measuring hematological parameter in patients with hepatitis B is an essential step in the knowledge of the affected state of the immune. The clinical application of these leukocyte morphologic parameters offers many advantages. These parameters are generated during automated differential analysis without additional specimen requirements [(238)]. Various kinds of hematological abnormalities have been known to occur in liver diseases, in viral hepatitis, bone marrow hypoplasia and pancytopenia had developed. In acute viral hepatitis, some abnormalities were observed such as anemia, thrombocytopenia, leucopenia, Aplastic anemia, and decrease in Haematocrit during the first three weeks of illness. This could be attributed to a temporary bone marrow suppression and autoimmune hemolytic anemia, which may accompany viral hepatitis [(239)]. In chronic liver disease, significant causes of anemia are hemorrhage due to impaired blood coagulation caused by blood coagulation factors deficiency and/ or thrombocytopenia [(237)].

3.6.2 Patients Distribution by Gender:

The patients were distributed into males and females revealed some significant differences in the level of RBC, HB, and HCT to HBV (HBeAg positive) patients. RBC level to females patients with (HBeAg positive) showed a significant decrease compared to male patients with (HBeAg positive) (4.60 ± 0.32 *vs.* 4.98 ± 0.50). HB level to females patients with (HBeAg positive) showed a significant decrease compared to male patients with (HBeAg positive) patients (12.45 ± 1.31 g/dl *vs.* 13.71 ± 1.72 g/dl). HCT level to females patients with (HBeAg positive) showed a significant decrease compared to male patients with (HBeAg positive) patients (39.68 ± 3.63 % *vs.* 43.29 ± 5.54%). In HBV (HBeAg negative) female patients showed a significant increase of fibrinogen and

PLT compared to males patients HBV (HBeAg negative), while level of RBC, HB, HCT, MCH, and MCHC showed a significant decrease in female HBV (HBeAg negative) patients compared to male HBV (HBeAg negative) patients as shown in table (3-17).

Table 3-17 Hematological investigations in hepatitis B patients according to gender.

Hematological Parameters	(HBeAg positve +ve) patients		(HBeAg negative) patients	
	Male	Female	Male	Female
Fibrinogen (mg/dl)	294.6 ± 85.0 a	258.2 ± 61.2 a	234.8 ± 66.6 b	298.5 ± 85.6 a
Prothrombin time (sec.)	14.86 ± 1.11 a	15.62 ± 2.55 a	16.29 ± 2.94 a	15.05 ± 1.17 a
INR	1.24 ± 0.16 a	1.37 ± 0.38 a	1.49 ± 0.45 a	1.29 ± 0.25 a
WBC *10^3	8.82 ± 2.64 a	7.85 ± 2.34 a	7.13 ± 2.06 a	7.78 ± 1.70 a
Lymphocyte %	33.32 ± 11.92 a	32.30 ± 9.80 a	30.47 ± 6.99 a	29.57 ± 9.75 a
Monocytes %	9.19 ± 2.32 a	8.78 ± 2.70 a	7.19 ± 3.272 a	8.572 ± 2.357 a
Granulocytes %	57.49 ± 13.08 a	58.91 ± 11.04 a	62.34 ± 8.11 a	61.86 ± 11.03 a
RBC *10^6	4.98 ± 0.50 a	4.60 ± 0.32 b	5.212 ± 0.543 a	4.67 ± 0.37 b
Hemoglobin (g/dl)	13.71 ± 1.72 a	12.45 ± 1.31 b	14.741 ± 1.310 a	12.21 ± 1.63 b
HCT (PCV) %	43.29 ± 5.54 a	39.68 ± 3.63 b	45.78 ± 3.8 a	40.13 ± 4.45 b
MCV (fl)	86.91 ± 6.36 a	86.285 ± 2.34 a	88.25 ± 6.36 a	86.00 ± 7.88 a
MCH (pg)	27.56± 2.03 a	27.09 ± 2.35 a	28.36 ± 2.29 a	26.14 ± 2.98 b
MCHC (g/dl)	31.72 ± 0.98 a	31.36 ± 1.21 a	32.15 ± 0.76 a	30.33 ± 1.31 b
Platelets *10^3	319.1 ± 103.4 a	286.1 ± 77.7 a	214.1 ± 68.1 b	264.7 ± 69.0 a

Data presented as Mean ± SD, * significant differences (*p*<0.05), (**) highly significant differences (*p*<0.01). †Means that do not share a letter (Horizontally) within the group are significantly different (according to Independent t-Test).

Both sexes can be affected by HBV at which the distribution results of study groups according to sex, the results showed that females are more affected by hematological parameters than males. The difference in exposure rate to HBV infection between both sexes may be

attributed to occupational factors and other risk factors associated with the exposure of females like physiological and hormonal differences between males and females.

3.6.3 Patients Distribution by Age Groups:

Table (3-18) shows the distribution of HBV (HBeAg positive) and HBV (HBeAg negative) patients and the comparison between them according to the age. Distribution patients into six age groups (< 21, 21-30, 31-40, 41-50, 51-60 years and > 60 years) revealed some significant differences between variables. In HBV (HBeAg positive) patients, lymphocyte % it was found the higher level in the age ranged < 21 years with a mean of (36.80±11.65), and the least level in the age ranged > 60 years with a mean of (17.15±1.63). Monocytes % it was found the higher level in the age ranged < 21 years with a mean of (10.12±1.90), and the least level in the age ranged 31-40 year with a mean of (6.80±1.91).Granulocytes % it was found the higher level in the age ranged > 60 years with a mean of (75.40±2.26), and the least level in the age ranged< 21 years with a mean of (53.07±12.55). MCV (fl) it was found the higher level in the age ranged > 60 years with a mean of (97.95±2.76), and the least level in the age ranged < 21 years with a mean of (83.72±3.64). MCH (pg) it was found the higher level in the age ranged > 60 years with a mean of (30.40±1.13) and the least level in the age ranged 31-40 year with a mean of (25.23±4.51). MCHC (g/dl) it was found the higher level in the age ranged < 21 years with a mean of (31.88±0.95) and the least level in the age ranged 31-40 year with a mean of (30.05±1.82). While fibrinogen, Prothrombin time, INR, WBC, RBC, HB, HCT, MCH, and Platelets showed no significant differences between different age groups.

In HBV (HBeAg negative) patients level of PT, It was found the higher level in the age ranged > 60 years with a mean of (25.4±0.00), and the least level in the age ranged 31-40 year with a mean of (14.85±1.4). INR It was found the higher level in the age ranged > 60 years with a mean of (2.70±0.00), and the least level in the age ranged 31-40 year with a mean of (1.27±0.29). While fibrinogen, WBC, Lymphocyte %, Monocytes %, Granulocytes %, RBC, HB, HCT, MCV, MCH, MCHC, and Platelets showed no significant differences between different age groups.

Table 3-18 Hematological investigations in hepatitis B patients according to age groups.

Hematological parameters	Age mean ± SD	(HBeAg positive) patients						(HBeAg negative) patients					
		< 21	21-30	31-40	41-50	51-60	> 60	< 21	21-30	31-40	41-50	51-60	> 60
Fibrinogen (mg/dl)	Mean	263.70 a	291.66 a	313.18 a	217.23 a	-	334.45 a	288 a	244 a	274 a	265 a	221 a	330 a
	SD	71.03	65.22	126.74	12.65	-	79.55	76.3	64.3	114.4	79.1	35.1	0.0
Prothrombin time (sec.)	Mean	15.16 a	15.08 a	14.15 a	17.93 a	-	15.05 a	15.16 c	15.47 c	14.88 c	15.60 c	19.35 b	25.40 a
	SD	1.20	1.83	1.17	5.39	-	1.48	1.34	1.58	1.40	2.15	2.47	0.00
INR	Mean	1.30 a	1.28 a	1.20 a	1.65 a	-	1.26 a	1.29 b	1.39 b	1.27 b	1.35 b	2.00 a	2.70 a
	SD	0.20	0.30	0.27	0.74	-	0.23	0.20	0.32	0.29	0.34	0.28	0.00
WBC *10^3	Mean	8.73 a	7.66 a	7.88 a	7.30 a	-	10.60 a	8.34 a	7.19 a	7.57 a	7.38 a	6.40 a	5.00 a
	SD	2.77	1.10	3.06	2.76	-	4.67	2.12	1.30	1.26	3.19	3.68	0.00
Lymphocyte %	Mean	36.80 a	28.43 bc	30.4abc	36.00 ab	-	17.15 c	35.09 a	27.27 a	29.18 a	29.85 a	28.20 a	48.00 a
	SD	11.65	8.21	5.74	7.11	-	1.63	5.22	8.58	8.77	6.29	4.24	0.00
Monocytes %	Mean	10.12 a	8.12 ab	6.80 b	8.50 ab	-	7.45 ab	9.46 a	7.75 a	7.61 a	7.58 a	5.00 a	6.20 a
	SD	1.90	2.48	1.91	4.64	-	0.64	1.87	3.68	2.55	2.53	3.54	0.00
Granulocytes %	Mean	53.07 b	63.46 a	62.85 ab	55.50 ba	-	75.40 a	55.46 a	64.98 a	63.21 a	62.57 a	66.80 a	45.80 a
	SD	12.55	8.37	7.11	10.14	-	2.26	6.51	9.75	10.15	6.70	7.78	0.00
RBC *10^6	Mean	4.70 a	4.82 a	5.12 a	4.86 a	-	4.80 a	5.15 a	4.96 a	4.79 a	5.24 a	4.83 a	4.25 a
	SD	0.44	0.33	0.71	0.80	-	0.02	0.72	0.53	0.52	0.27	0.49	0.00
Hemoglobin (g/dl)	Mean	12.50 a	13.66 a	13.08 a	13.77 a	-	14.60 a	13.21 a	13.61 a	12.94 a	15.27 a	14.20 a	11.70 a
	SD	1.13	1.26	3.66	1.86	-	0.57	1.48	2.22	2.07	0.58	0.85	0.00
HCT (pcv) %	Mean	39.31 a	43.27 a	43.08 a	43.67 a	-	47.00a	42.66 a	43.36 a	41.55 a	47.38 a	44.00 a	36.10 a
	SD	3.71	3.49	10.07	5.35	-	1.56	3.09	5.77	5.44	1.80	1.98	0.00
MCV (fl)	Mean	83.72 c	89.86 b	83.45 c	90.47 ab	-	97.95 a	83.66 a	87.46 a	86.73 a	90.65 a	91.50 a	85.10 a
	SD	3.64	3.78	11.00	6.96	-	2.76	8.38	8.02	6.67	3.62	5.37	0.00
MCH (pg)	Mean	26.69 bc	28.38 ab	25.23 c	28.47 ab	-	30.40 a	25.86 a	27.37 a	26.92 a	29.13 a	29.40 a	27.50 a
	SD	1.48	1.38	4.51	0.93	-	1.13	2.85	3.23	2.81	1.26	1.27	0.00
MCHC (g/dl)	Mean	31.88 a	31.57 ab	30.05 b	31.53 a	-	31.00 ab	30.91 a	31.24 a	30.98 a	32.15a	32.20 a	32.40 a
	SD	0.95	0.69	1.82	1.36	-	0.28	1.38	1.71	1.27	0.24	0.42	0.00
Platelets *10^3	Mean	319.85 a	258.64 a	307.00 a	327.00 a	-	327.0 a	262.0 a	220.5 a	260.6 a	228.6 a	199.5 a	177.0 a
	SD	94.21	53.80	48.42	152.50	-	217.79	73.83	70.72	75.50	54.42	139.30	0.00

® Means that do not share a letter are significantly different horizontally within each group with (P<0.05), according to the least significant difference (LSD)

These results have coincided partially with other studies carried out in Iraq such as (AL-Khilkhali) [238], who reported that the subjects of this study are investigated the importance of measuring the blood parameters in hepatitis B patients, and detection of the patient's immune response against viral infection. The obtained results showed that 50 were seropositive hepatitis B in ELISA technique. The age groups (32-41) and (42-51) years showed a highly significant ($P<0.05$) elevation in patients than other patients. In contrast white blood cell count indicate that the age groups (12-21) and (42-51) years recorded high significant difference ($p<0.05$) compared with healthy groups and other patients while Lymphocyte and monocyte percentage increase in more groups of patients suffering from hepatitis B compared with healthy control group-specific in age groups (42-51) and (52-61) years in comparison to other patients. On the other hand, the result of granulocytes, the basophile showing high significant ($p<0.05$) increase in patients group as compared to the healthy control group and other granulocytes. The other study carried out in Nigeria whose results revealed that there was a significant difference between the hemoglobin concentration, monocytes and packed cell volume of hepatitis B infected subjects of different age group ($P<0.05$) [239].

Several studies have focused on the relationship between viral hepatitis and hematological parameter, which has been required for viral clearance, is produced in response to viral infection [231, 240]. This variation in the results may be due to the following factors: the first patients in our study are of different age than those in the other research. Secondly, the geographic variation in HBV genotypes in the study population would influence the prevalence of hepatitis B. Third factor

was that the cause might also be nutritional or infectious. Finally, the difference is in sample size.

3.7 Correlation of biomarkers:

3.7.1 Correlation among biomarkers in HBV (HBeAg positive) and HBV (HBeAg negative) Patients:

Pearson correlation applying on blood biomarkers in HBV (HBeAg positive) patients, demonstrated in the table (3-19), found a significant negative relationship between RT-PCR level and each of HB, HCT, and MCV levels (r=-0.33,r=-0.37,r=-0.35) respectively and negative correlation between V.C level and the level of IL-2(r=-0.31). Additionally, there is a strong negative correlation between SOD level and IL-1 alpha level (r=-0.41). On the other hand, there is a significant negative correlation between MDA level and each of ALP, Monocytes %, and Platelets levels (r=-0.36, r=-0.32, r=-0.35) respectively, and strong negative relationship between MDA level and the level of WBC(r=-0.38) while there is a significant positive correlation between IL-2 level and the level of WBC (r=0.34) and strong negative correlation between IL-2 and Monocytes %(r=-058).

Pearson correlation applying on blood biomarkers in HBV (HBeAg negative) patients, explained in table (3-20), observed that there is significant strong negative correlation between HBS Ag level and MDA level (r=-0.47), while there is significant negative correlation with V.C level (r= -0.33) and IL-2 level (r= -0.33)and INR level(r= -0.31). Additionally, there is a strong positive correlation between HBS Ag level and fibrinogen levels (r=0.38), and there is a significant positive correlation between HBS Ag level and each of WBC and Monocytes % levels (r= 0.31, r=0.34) respectively.

Furthermore, the current result reveals that RT-PCR level which was positively correlated with each of Albumin and Albumin/Globulin (r= 031, r= 0.38) respectively and significant strong negative correlation with Monocytes % levels (r= -0.47) while there is a negative correlation between RT-PCR level and fibrinogen (r= -0.35).

On the other hand, this study records a significant strong negative correlation between V.C level and each of IL-2, TP, Albumin, and Globulin levels (r=-0.38, r=-0.47, r=-0.40, r=-0.38) respectively. While there is a strong positive correlation between V.C level and each of SOD, MDA, and Indirect Bilirubin levels (r=0.44, r=0.71, r=42) respectively.

Regarding the SOD serum level, there is a significant positive correlation with MDA level (r=0.34).

Additionally, there is a strong positive correlation between MDA level and each of TSB and Indirect Bilirubin levels (r=0.56, r= 0.46) respectively.

On the other hand, this study records a significantly strong negative correlation between IL-1 alpha level and Albumin/Globulin level (r= -0.39).

Remarkably, IL-2 correlate strong significantly with each of Direct Bilirubin, Total protein, INR, and MCHC(r=0.49, r=0.45, r=0.41, r=0.39) respectively. And correlate significantly with each of ALT(r= -0.32), GGT (r= -0.35), and Prothrombin time (r= 0.32). While there is significant strong negative correlation with Fibrinogen level (r= -0.43) and Monocytes % level (r= -0.63).

Table 3-19 Correlation of study biomarkers in HBV (HBeAg positive) patients

	HBS Ag level (IU/ml)	HBV-DNA (IU/ml)	V.C (ng/ml)	SOD (U/L)	MDA (n mol/ml)	IL-1 alpha (pg/ml)	IL-2 (pg/ml)
HBV-DNA IU/ml	0.10						
V.C ng/ml	-0.15	-0.22					
SOD U/L	-0.03	0.00	-0.11				
MDA n.mol/ml	-0.05	-0.21	0.28	0.08			
IL-1 alpha pg/ml	0.12	-0.12	0.16	-0.41**	0.06		
IL-2 pg/ml	0.15	0.29	-0.31*	0.10	0.15	-0.01	
AST I.U/L	0.14	-0.07	0.01	-0.15	-0.14	0.20	-0.13
ALT I.U/L	0.12	-0.10	0.00	-0.16	-0.13	0.19	-0.11
GGT I.U/L	0.17	-0.24	-0.01	-0.22	0.01	0.22	0.04
ALP I.U/L	0.05	0.21	-0.19	-0.18	-0.36*	0.14	-0.27
T.S.B mg/dl	0.15	-0.13	-0.05	-0.14	-0.15	0.18	-0.13
Direct Bilirubin mg/dl	0.11	-0.12	-0.03	-0.10	-0.07	0.21	0.01
Indirect Bilirubin mg/dl	0.17	-0.13	-0.07	-0.16	-0.21	0.14	-0.24
Total protein g/dl	0.28	0.16	-0.03	-0.20	0.17	0.28	0.01
Albumin g/dl	0.20	0.23	0.02	-0.19	0.28	0.28	0.07
Globulin g/dl	0.30	0.08	-0.06	-0.18	0.05	0.24	-0.04
Albumin/Globulin	-0.24	0.01	0.12	0.06	0.17	-0.03	0.11
Fibrinogen mg/dl	-0.02	0.06	0.15	-0.22	-0.11	-0.03	0.12
Prothrombin Time (sec)	0.10	0.25	0.00	-0.16	-0.13	0.18	0.07
INR	0.06	0.29	0.04	-0.18	-0.12	0.19	0.09
WBC *10^3	0.09	0.23	-0.24	-0.06	-0.38**	-0.04	0.34*
Lymphocyte %	-0.01	0.15	0.12	0.15	-0.01	-0.16	-0.02
Monocytes %	-0.18	0.07	0.12	0.07	-0.32*	-0.08	-0.58**
Granulocytes %	0.05	-0.15	-0.14	-0.15	0.08	0.16	0.14
RBC *10^6	-0.15	-0.19	0.13	-0.07	0.13	0.30	0.15
Hemoglobin g/dl	-0.20	-0.33*	0.02	-0.02	0.27	0.24	0.24
HCT (pcv) %	-0.18	-0.37*	0.09	-0.06	0.25	0.26	0.17
MCV fl	-0.12	-0.35*	-0.06	0.00	0.23	0.05	0.10
MCH pg	-0.16	-0.27	-0.15	0.06	0.26	0.01	0.19
MCHC g/dl	-0.11	0.11	-0.26	0.13	0.11	-0.07	0.27
Platelets *10^3	0.10	0.25	0.08	-0.20	-0.35*	-0.02	0.06

* Significant (P < 0.05) ** Significant (P < 0.01)

Table 3-20: Correlation of study biomarkers in HBV (HBeAg negative) patients

	HBS Ag level IU/ ml	HBV-DNA IU/ ml	V.C ng/ ml	SOD U/L	MDA n. mol/ ml	IL-1 alpha pg/ ml	IL-2 pg/ml
HBV-DNA IU/ml	-0.07						
V.C ng/ml	-0.33*	-0.17					
SOD U/L	-0.25	-0.11	0.44**				
MDA n.mol/ml	-0.47**	-0.12	0.71**	0.34*			
IL-1 alpha pg/ml	-0.04	-0.2	-0.07	0.05	0		
IL-2 pg/ml	-0.33*	0.14	-0.38**	-0.13	-0.02	-0.07	
AST I.U/L	0.07	0.08	-0.26	-0.14	0.04	-0.16	0.22
ALT I.U/L	-0.04	0.19	-0.29	-0.13	-0.03	-0.16	0.32*
GGT I.U/L	-0.10	0.15	-0.20	-0.22	0.21	-0.19	0.35*
ALP I.U/L	0.1	-0.16	-0.04	0.07	-0.13	-0.03	-0.21
T.S.B mg/dl	-0.29	0.09	0.3	0.06	0.56**	-0.12	0.28
Direct Bilirubin mg/dl	-0.27	0.07	-0.15	0.04	0.29	-0.1	0.49**
Indirect Bilirubin mg/dl	-0.17	0.06	0.42**	0.04	0.46**	-0.07	0.02
Total protein g/dl	-0.05	0.12	-0.47**	-0.04	-0.26	-0.02	0.45**
Albumin g/dl	-0.14	0.31*	-0.40**	-0.03	-0.14	-0.23	0.6
Globulin g/dl	0.06	-0.12	-0.38**	-0.04	-0.3	0.22	0.11
Albumin/Globulin	-0.16	0.38*	0.12	0.06	0.22	-0.39**	0.28
Fibrinogen mg/dl	0.38**	-0.35*	0.22	0.28	0.07	-0.03	-0.43**
Prothrombin Time (sec)	-0.22	0.03	-0.17	-0.06	0.28	-0.17	0.32*
INR	-0.31*	0.06	-0.17	-0.07	0.28	-0.21	0.41**
WBC *10^3	0.31*	0.14	-0.01	-0.09	-0.22	-0.05	-0.05
Lymphocyte %	0.1	-0.15	-0.24	-0.1	-0.06	-0.06	0.14
Monocytes %	0.34*	-0.47**	0.24	0.14	0	0.02	-0.63**
Granulocytes %	-0.2	0.28	0.13	0.04	0.06	0.04	0.08
RBC *10^6	-0.11	0.02	0.18	-0.11	0.06	0.01	-0.2
Hemoglobin g/dl	-0.15	0.18	0.18	-0.19	0.16	0.06	-0.02
HCT (pcv) %	-0.1	0.11	0.22	-0.19	0.15	0.13	-0.17
MCV fl	0.05	0.10	0.05	-0.13	0.12	0.15	0.02
MCH pg	-0.06	0.20	0.04	-0.15	0.15	0.05	0.17
MCHC g/dl	-0.22	0.30	0.00	-0.13	0.13	-0.14	0.39**
Platelets *10^3	-0.08	0.01	0.10	0.10	-0.08	-0.16	-0.23

* Significant ($P < 0.05$) ** Significant ($P < 0.01$)

CONCLUSIONS:

The study concludes the following:

1- qRT-PCR test have the superior clinical advantage among other techniques, and it is highly recommended for practical application for the diagnosis and follow up of HBV-DNA infection through detection the exact number of viral DNA copies.

2-The biochemical parameters, especially (ALT, AST, GGT, and ALP), were elevated in HBV patients significantly, where the result showed both (AST, ALT, and ALP) of HBeAg positive patients significantly increased compared to HBeAg negative patients and healthy control, while the GGT showed a significant increase in each HBeAg positive and HBeAg negative patients compared to healthy control. This gives us an indication of the weakness performance of the liver of patients with hepatitis B.

3-The activities of the non-enzymatic anti-oxidant system, including vitamin C, were significantly decreased in HBeAg positive patients in comparison with HBeAg negative patients and healthy control. Also, enzymatic anti-oxidants system, including superoxide dismutase, was significantly reduced in each HBeAg positive and HBeAg negative patients compared to healthy control.

4-The production of lipid peroxidation marker malondialdehyde has been markedly reduced in HBV patients.

5-There was no significant difference between serum levels of (IL-1 alpha) in patients and controls, while serum levels of (IL-2) was decreased in HBeAg positive patients group relative to HBeAg negative patients.

6-The hematological parameters (PT, INR, PCV, and PLT) for hepatitis B patients showed a significant increase, while a significant decrease

was found in (Monocytes %, Granulocytes %, RBC, and MCHC) parameters in hepatitis B patients.

7-The results suggest a highly significant negative correlation between superoxide dismutase and IL-1 alpha concentration in HBeAg positive patients and the negative relationship between vitamin C and IL-2 concentration in HBeAg positive patients.

8-Serum vitamin C levels showed a highly positive correlation with superoxide dismutase, malondialdehyde, and highly negative correlation with IL-2. Also, superoxide dismutase showed a positive correlation with malondialdehyde; on the other hand, IL-2 showed a positive correlation with ALT, TP, and negative correlation with Fibrinogen in HBeAg negative patients.

Conclusions and Recommendation

RECOMMENDATIONS:

1- The need for further studies, comprising a larger sample to asses possible association between the presence of histopathological status, viral genotypes, and even in patients without evidence of circulating antibodies.

2-Cytokine gene polymorphisms are fruitful area of research that can aid in a further understanding of hepatitis immunogenetic predisposition, and other cytokines, which are not investigated in the present study; for instance IL-17 family, IL-18, IL-31, and IL-33 represent a good target for research in hepatitis in terms of their serum levels and genotypes.

3-Conduct an epidemiological study to shed more light on the sources of the HBV infection in Anbar Province and follow the suitable strategies for the prevention of the infection and apply effective treatment regimens.

4- More studies should be conducted to compare between intracellular and extracellular antioxidant by isolation of RBCs from different types of HBV in addition to study the relationship between age, BMI, and the levels of other antioxidant parameters such as GPX, CAT, vitamin A, and E and selenium.

5- Studying the effect of the antioxidant supplement on HBS Ag levels in experimental animals.

6- Use of cytokine antibody array to identify and describe the relationships between the other varieties of human cytokines and signaling pathways.

7- Using other parameters with different samples (such as a serum, saliva, and urine) to evaluate the progressing of hepatitis B virus, like

adiponectin and different inflammatory cytokines (IL-6, and TNF-α) and study the correlation between these parameters.

8- It is essential to correlate the viral burden with the immune activity such as INF alpha and beta, and counting of T-cells spreading (CD4+ and CD8+).

References

References:

1. Jefferies, M., Rauff, B., Rashid, H., Lam, T., and Rafiq, S. (2018). Update on global epidemiology of viral hepatitis and preventive strategies. World J Clin Cases November 6, 6(13), 589-599.

2. Abdullah, S.M. (2018). Prevalence of Hepatitis B and C virus infection and their co-relation with hematological and hepatic parameters in subjects undergoing Premarital Screening in the Jazan Region, Kingdom of Saudi Arabia. Pak J Med Sci, 34(2), 316-321.

3. Mitra, B., Thapa, R.J., Guo, H., and Block, T.M. (2018). Host functions used by hepatitis B virus to complete its life cycle:Implications for developing host-targeting agents to treat chronic hepatitis B. Antiviral Research, 158 ,185–198.

4. Meireles, L.C., Marinho, R.T., and Damme, P.V. (2015). Three decades of hepatitis B control with vaccination. World J Hepatol, 7(18): 2127-2132.

5. Al-Suraifi, A.S.K., Al-Rubaie, A.D.J., Al-Mayahie, S.M.G., and Al-Abedy, N.M.M. (2016). Unusual HBV Mixed Genotype Infections Among Hepatitis Type B Iraqi Patients in Wasit Province/Iraq. International Journal of Biomedical Engineering and Clinical Science, 2(1): 1-7.

6. Kolawole, O.M., Kana, S.N. (2018). Hepatitis B Virus and Malaria Co-infection Causing Significant Changes in Hematological and Liver Function Indices in a Cohort of Subjects in Ilorin, Nigeria. Int J Infect. July; 5(3):e81528.

7. Brown, R.S.J.r., McMahon, B.J., Lok, A.S., Wong, J.B., and Ahmed, A.T.,et.al. (2016). Antiviral therapy in chronic hepatitis B viral infection during pregnancy: A systematic review and meta-analysis. Hepatology. Jan;63(1):319-333.

8. Al-Hamdani, A.H., Al-Rawy, S.K., and Khamees, H.A. (2012). Retrospective Seroprevalence Study of Hepatitis B and C in Iraqi Population at Baghdad: A Hospital Based Study. Iraqi J. Comm. Med., July. (3): 186-190.

9. Tong, M.J., Pan, C.Q., Hann, H., Kowdley, V. K., Han, S.B., Min, A.D., and Leduc, T. (2011). The Management of Chronic Hepatitis B in Asian Americans. J. Dig Dis Sci. 56:3143–3162.

10. Mauss, S., Berg, T., Rockstroh, J., Sarrazin, C., and Wedemeyer, H. (2018). HEPATOLOGY A clinical textbook 9th Edition; pp: 151-153, 39.

11. Fattovich, G., Giustina, G., Realdi, G., Corrocher, R., and Schalm, S.W. (1997). Longterm outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. European Concerted Action on Viral Hepatitis (EUROHEP). J. Hepatology 26:1338-1342.

12. Mendy, M.E., Kaye, S., van der Sande, M., Solon, P.R., Waight, P.A., Shipton, D., Awi, D., Snell, P., Whittle, H., and McConkey, S.J. (2006). Application of real-time PCR to quantify hepatitis B virus DNA in chronic carriers in The Gambia. J. Virology Journal, 3:23-29.

13. Al-Qaysi, S.A.A. (2012). Molecular and Serological Study of Hepatitis B Virus from Carriers at Al-Ramadi City. A Thesis

Submitted to the Council of the College of Medicine University of Anbar in Partial Fulfillment of the Requirements for the degree of Master of Science in Medical Microbiology.

14. Cornberg, M., Protzer, U., and Petersen, J. (2011). Aktualisierung der S3 Leitliniezur Prophylaxes, Diagnostic und Therapy der Hepatitis-B-Virus infection. J. Gastroenterology, 49:871-930.

15. Rowicka, G., Dyląg, H., Ambroszkiewicz, J., Riahi, A., Weker, H., and Chełchowska, M. (2017). Total Oxidant and Antioxidant Status in Prepubertal Children with Obesity. Oxidative Medicine and Cellular Longevity, 6 pages.

16. Rahal, A., Kumar, A., Singh, V., Singh, B. S., Tiwari, R., Chakraborty,S., and Dhama, K. (2014). Oxidative stress, prooxidants, and antioxidants: The interplay. BioMed Research International, 2014:1-19.

17. Aliahmat, N. S., Noor, M. R. M., Yusof, W.J. W., Makpol ,S., Ngah, W.Z.W., and Yusof, Y. A. M . (2012). Antioxidant enzyme activity and malondialdehyde levels can be modulated by Piper bet, tocotrienol rich fraction and C hlorella vulgaris in aging C57BL/6 mice. CLINICS 67:1447-1454.

18. Andonova, L., Georgieva, M., and Zlatkov, A.l. (2015). Free radicals, oxidative stress and diseases associated with them PHARMACIA . 62 : 26 – 39.

19. Ferhan, M. A. M. (2016). Evaluation of Some Oxidant – Antioxidant Parameter and Mitochondrial Cytochrome C-1 Oxidase Gene in Smokers' Secondary Polycythemic Men A Thesis Submitted to the Council of the College of Science for Women ,University of Babylon

in Partial fulfillment of the requirements for the Degree of Master of Science in Biology (Zoology).

20. Kshitiz, K. K., Varun, S. K., Ranjan, A., and Kesari J.R. (2015). Study of serum malondialdehyde and vitamin c status in type 2 diabetes mellitus.INT.J.Curr.Res.Aca.Rev, 3(4):20-25.

21. Kurutas, E. B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition journal, 15(1), 71.

22. Mehde, A. A., Mehdi, W. A., and Jasim, A. M. (2013). Study Several Biochemical Parameters into Patients with Hepatitis B Virus. Global Journal of Medical Research, 13(2), 22.

23. Di Meo, S., Reed, T. T., Venditti, P., and Victor, V. M. (2016). Role of ROS and RNS sources in physiological and pathological conditions. Oxidative Medicine and Cellular Longevity, 2016.

24. Chokkalingam, V., Tel, J., Wimmers, F., Liu, X., Semenov, S., Thiele, J., Figdor, C. and Huck, W. T. (2013). Probing cellular heterogeneity in cytokine-secreting immune cells using droplet-based microfluidics. Lab Chip, 24:4740-4744.

25. Rhoades, R. A, Bell, D. R. (2013). Medical physiology principles for clinical medicine. Fourth edition.

26. Abbas, A.H. (2017). Relationship of Vitamin D with α-Fetoprotein, interlukin-17 and interferon-γ in chronic viral hepatitis patients. A Thesis submitted to the college council of health and medical technology as a partial fulfillment of the requirements for the master of technology in medical laboratory science.

27. AL –Janabi, W.M.S. (2015). Estimation of Some Elements Concentration in Patients Hepatitis B in Fallujah District. A Thesis Submitted to the Council of College of Science University of Anbar As Partial Fulfillment for the Requirement of the PhD Degree of Science in Chemistry.

28. Obead, W.K. (2012). Glycoproteins, leptin & some Biochemical Markers in Sera of patients with viral hepatitis B&C. A thesis Submitted to the College of Science at the University of AL-Mustansiriya as a Partial Fulfillment of the Requirements for the Degree of Master of Science in Biochemistry.

29. College, N.R, Walker, B.R., and Ralston, S.H. (2010). Davidson's principles & practice of medicine. 21st ed.,pp: 449,922-954, 924-926, 947,925.

30. Stevens, C.D. (2010). Clinical immunology and serology. A laboratory perspective. Third edition by F.A.Davis Company. Philadelphia.

31. Muhammad, G., and Muhammad, K., P. (2009). Identification of epidemiological risk factor for hepatitis C In Punjap Pakistan, Journal of Ayub Medical College Abbottabad. 21(2):156-161.

32. Mauss, S., Berg, T., Rockstroh, J., Sarrazin, C., and Wedemeyer, H. (2010). HEPATOLOGY A clinical textbook second Edition; pp: 11-12.

33. World Health Organization (2018). Hepatitis B. World Health Organization. Collected 18 July 2018 from https://www.who.int/news-room/fact-sheets/detail/hepatitid-b.

34. Wang, S., Tao, Y., Tao, Y., Jiang, J., Yan, L., Wang, C., et al. (2018). Epidemiological study of hepatitis B and hepatitis C infections in Northeastern China and the beneficial effect of the vaccination strategy for hepatitis B: a cross-sectional study. BMC Public Health. 3; 18(1):1088. doi: 10.1186/s12889-018-5984-6.

35. Xiao, L., Xian, J., Li, Y., Geng, A., Yang, X., Han, L., and Xu, H. (2014). Parameters Associated with Significant Liver Histological Changes in Patients with Chronic Hepatitis B. Hindawi Publishing Corporation ISRN Gastroenterology Volume 2014, Article ID 913890, 6 page.

36. Baghbanian, M., Halvani, M., Roghani, H.S., Lotfi, M.H., Yazdi, M.F., and Vahedian-Ardakani, H.A. (2016). PREVALENCE OF OCCULT HEPATITIS B INFECTION IN IRANIAN CANCER PATIENTS BEFORE CHEMOTHERAPY TREATMENT. Arq Gastroenterol. Jul-Sep; 53(3):175-9.

37. Vasmehjani, A., Yaghoubi, S., Hashemi, S., Farahmand, M., Adeli,. O, Am Taravand, A., and Beiranvand, M. (2018). The Prevalence of Hepatitis B, Hepatitis C, and Human Immunodeficiency Virus Infections among β-thalassemia Major: A Multicenter Survey in Lorestan, West of Iran. Iran J Ped Hematol Oncol, Vol 8.No 2, 111-117.

38. Mauss, S., Berg, T., Rockstroh, J., Sarrazin, C., & Wedemeyer, H. (2017). HEPATOLOGY A clinical textbook 8th Edition.

39. World Health Organization**.** (2015). Guidelines for the Prevention, Care, and Treatment of Persons with Chronic Hepatitis B Infection.

Available at: http://apps. who.int/medicinedocs/documents/ s21813 en/ s21813en.pdf.

40. Mauss, S., Berg, T., Rockstroh, J., Sarrazin, C., and Wedemeyer, H. (2014). HEPATOLOGY A clinical textbook fifth Edition; pp: 37

41. Kamil, Y.M. (2014). Study of some biological variables in patients with hepatitis B and C. A Thesis Submitted to the Council of College of Science, Al-Mustansiriyah University in Partial Fulfillment of the Requirement for the Degree of Master of Zoology.

42. Ryan, K.J., Ray, C.G., Ahmad, N., Drew, W.L., Lagunoff, M., Pottinger, P., Reller, L.B., and Sterling, C.R. (2014). Sherris Medical Microbiology. 6th ed. United State of America: McGraw Hill Companies; 228- 237.

43. Lavanchy, D., and Kane, M. (2016). Global epidemiology of hepatitis B virus infection. In Hepatitis B Virus in Human Diseases (pp: 187-203). Springer International Publishing.

44. Nagpal, B., Hegde, U., and Kulkarni, M. (2017). Seroprevalence of hepatitis B antigenemia among dental students in a private South Indian dental institution. International Journal of Research in Medical Sciences, 3(12), 3543-3547.

45. Brousseau, N., Murphy, D. G., Gilca, V., Larouche, J., Mandal, S., and Tedder, R. S. (2017). Acute hepatitis B virus infection with delayed appearance of hepatitis B core antibody in an immunocompromised patient: a case report. Journal of Medical Case Reports, 11(1), 111.

46. Kadham, M.J. (2018). Precore and Basal Core Promoter mutations of chronic Hepatitis B virus in relation to drug resistance chronic

infections in some Iraqi patients. Mustansiriyah University in partial fulfillment of the degree of Doctorate of Philosophy in Biology / Microbiology.

47. Gunardi, H., Iskandar, M. Y., Ie, S. I., Dwipoerwantoro, P. G., Gani, R. A., and Muljono, D. H. (2017). Hepatitis B virus infection in children of HBV-related chronic liver disease patients: a study of intra-familial HBV transmission. Hepatology International, 11(1), 96-104.

48. Khalil, A. S., Abdulkareem, W. L., and Hussein, N. R. (2016). The Effectiveness of Post-Exposure Prophylaxis in Infants Born to Hepatitis B Virus Positive Mothers in the Kurdistan Region, Iraq. International Journal of Infection.

49. Wong, D. H., Fung, J., Lee, C. K., Seto, W. K., Leung, J., Huang, F. Y. and Yuen, M. F. (2016). Intrahepatic hepatitis B virus replication and liver histology in subjects with occult hepatitis B infection. Clinical Microbiology and Infection, *22*(3), 290-e1-290-e3.

50. Suspène, R., Thiers, V., Vartanian, J. P., & Wain-Hobson, S. (2016). PCR mediated recombination impacts the analysis of hepatitis B Virus covalently closed circular DNA. Retrovirology, 13(1), 84.

51. Cai, C. W., Lomonosova, E., Moran, E. A., Cheng, X., Patel, K. B., Bailly, F., and Tavis, J. E. (2014). Hepatitis B virus replication is blocked by a 2-hydroxyisoquinoline-1, 3 (2H, 4H) - dione (HID) inhibitor of the viral ribonuclease H activity. Antiviral research, 108, 48-55.

52. Jonaidi-Jafari, N., Rezaee-Zavareh, M.S., Tavallaei-Nosratabadi, J., Ajudani, R., Ramezani-Binabaj, M., Karimi-Sari, H., Izadi, M., et al.

(2017). Occult Hepatitis B Infection in Hepatitis C Patients with Hematological Disorders. Jundishapur J Microbiol. February; 10(2):e40816.

53. Price, H., Dunn, D., Zachary, T., Vudriko, T., Chirara, M., Kityo, C. and Kaleebu, P. (2017). Hepatitis B serological markers and plasma DNA concentrations. AIDS (London, England), 31(8), 1109.

54. Towell, V., and Cowie, B. (2012). Hepatitis B serology. Australian family physician, 41(4), 212.

55. Di Bisceglie, A. M., Lombardero, M., Teckman, J., Roberts, L., Janssen, H. L. A., Belle, S. H., & Hoofnagle, J. H. (2017). Determination of hepatitis B phenotype using biochemical and serological markers. Journal of viral hepatitis, 24(4), 320-329.

56. Maini, M. K., and Bertoletti, A. (2017). HBV in 2016: Global and immunotherapeutic insights into hepatitis B. Nature Reviews Gastroenterology & Hepatology. 2017 Feb; 14(2):71-72.

57. Bertoletti, A., and Ferrari, C. (2016). Adaptive immunity in HBV infection. Journal of hepatology, 64(1), S71-S83.

58. Giersch, K., Allweiss, L., Volz, T., Dandri, M., and Lütgehetmann, M. (2017). Serum HBV pgRNA as a clinical marker for cccDNA activity. Journal of hepatology, 66(2), 460-462.

59. David, G., Ginzinger, D.G., (2002). Gene quantification using real-time quantitative PCR: An emerging technology hits the mainstream. Experimental Hematology 30: 503–512.

60. Al-Hamzawi, S.F. (2014). Immunological Status for Hepatitis B and C Virus, IVS1-110, and Cd39 Mutations Frequencies among β-

Thalassemia patients in Diwaniya. A Thesis Submitted to the Council of the College of Medicine / University of Al-Qadisiya for in Partial Fulfillment of the Requirements the Degree of Master of Science in Medical Microbiology.

61. Liu, W. C., Mizokami, M., Buti, M., Lindh, M., Young, K.C, and Sun, K.T. (2006). Simultaneous quantification and genotyping of hepatitis B virus for genotypes A to G by real-time PCR and two-step melting curve analysis. J Clin Microbiol 44:4491-7.

62. Yeh, S. H., Tsai, C. Y., Kao, J. H., Liu, C. J., Kuo, T. J., and Lin, M. W. (2004). Quantification and genotyping of hepatitis B virus in a single reaction by real-time PCR and melting curve analysis. J Hepatol 41:659-66.

63. Ahmed, A. M. (2013). Determination of Hepatitis B Virus Genotypes among Iraqi Chronic Hepatitis B Patients and Inactive HBV Carriers. Ph.D. Thesis. The genetic engineering and biotechnology institute.195pp.

64. Payungporn, S., Tangkijvanich, P., Jantaradsamee, P., Theamboonlers, A. and Poovorawan, Y. (2004). Simultaneous quantitation and genotyping of hepatitis B virus by real-time PCR and melting curve analysis. J Virol Methods 120:131-40.

65. Schiff, E.R., Sorrell, M.F., Maddrcy, and Willis, C. (2017). Schiffs Diseases of the Liver. 12th ed; 2: pp.752.-820.

66. Burtis, C.A., Ashwool, E.R., and Bruns, D.E. (2006). Tietz Textbook Clinical Chemistry and Molecular Diagnostics. 4th ed. Elsevier Inc; p. 1777.

67. Thapa, B.R., and Walia, A. (2007). Liver Function Tests and their Interpretation Indian Journal of Pediatrics; 74(Issue 7), 663-671.

68. Zainal, I.G., Safaa,A.A., and Wajeeh,K.O. (2013). Biochemical Parameters in Relation to Serum Alphafetoprotein & Leptin levels in Iraqi Pateints with Chronic Liver Diseases.Research Article; 3(1) :16-22.

69. Suljevic, D., Mehinovic, L., and Alijagic, A. (2016). Hepatitis and biochemical markers in correlation with alpha-fetoprotein as a diagnostic indicator for the HBV and HCV differentiation. Alban Med J; 3:13-20.

70. Wang, C.S. (2012). Impact of increasing alanine aminotransferase levels within normal range on incident diabetes. J Formos Med Assoc; 111 (4): 201-8.

71. Rigvardhan, S. K., Chopra, G.S., Negi, R., and Kotwal, A. (2016). Correlation of serological, Biochemical and molecular viral markers with histological parameters in chronic hepatitis B for assessment of response to therapy. International Journal of contemporary Medical Research. Volume 3 issue 8 August 2016.

72. Ghouri, N. (2010). Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. Hepatology; 52 (3): 1156-61.

73. Almo, S.C., Smith, D.L., Danishefsky, A.T., and Ringe, D. (1994). The structural basis for the altered substrate specificity of the R292D active site mutant of aspartate aminotransferase from E. coli. Protein Eng; 7 (3): 405–412.

74. Chelebi, N.A. (2016). Enzymatic Alteration in Hepatitis-B patients Within Kerkuk Province, Iraq. International Journal of Current Microbiology and Applied Sciences.ISSN:2319-7706 Volume 5 Number 7: pp. 854-862.

75. Gaze, D.C. (2007). The role of existing and novel cardiac biomarkers for cardioprotection. Curr. Opin. Invest. Drugs; 8 (9): 711–7.

76. Marbut, S.M.M. (2018). Serological diagnosis of hepatitis B and C in patients with chronic diseases in Baghdad city. A Thesis submitted to the council of college of Medicine / Tikrit University in partial fulfillment of the requirements for the degree of master in Medical Microbiology.

77. Hussein, R.H. (2011). Comparison in some biochemical and haematological tests between chronic hepatitis B and C. J. Pure App1. SCi; 24:155-62.

78. Mastoi, A.A., Devrajani, B.R., Shah, S.Z., Rohopoto, Q., Memon, S.A., Baloch, M., Qureshi, G.A., and Sami, W. (2010). Metabolic investigations in patients with hepatitis B and C. World J Gastroenterol. 2010 Feb 7; 16(5):603-607.

79. Prajapatia, D., Desai, K. Patil, S.N., & Chaudhari, S.V. (2017). ALKALINE PHOSPHATASE: SIGNIFICANCE IN DAIRY INDUSTRY. INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT. 4(12): December, 2017. ISSN: 2349-5197. Impact Factor: 2.715.

80. Sharma, U., Pal, D., and Rajendra, P. (2014). Alkaline Phosphatase: An Overview. Ind J Clin Biochem (July-Sept 2014) 29(3):269–278.

81. Pinart, M., Kunath, F., Lieb, V., Tsaur, I., Wullich, B., and Schmidt, S. (2018). German Prostate Cancer Consortium (DPKK). Prognostic models for predicting overall survival in metastatic castration-resistant prostate cancer: a systematic review. World J Urol. 2018 Dec 15; [PubMed].

82. Alvarez, A.M., Mukherjee, D., F.A.C.C. (2011). Liver Abnormalities in Cardiac Diseases and Heart Failure. INTERNATIONAL JOURNAL OF ANGIOLOGY/VOLUME 20, NUMBER 3.

83. Al-Janabi, M.S.I. (2018). Study the Effect of Some Enzymes and Biochemical Parameters on the Level of PSA in Iraqi Men. A Dissertation Submitted to The Council of College of Science of Anbar University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biochemistry.

84. Whitfield, J. B. (2001). Gamma glutamyl transferase. Critical reviews in clinical laboratory sciences, 38(4), 263-355.

85. Everhart, J. E., and Wright, E. C. (2013). Association of γ-glutamyl transferase (GGT) activity with treatment and clinical outcomes in chronic hepatitis C (HCV). Hepatology, 57(5), 1725-1733.

86. United Healthcare. (2018). GAMMA GLUTAMYL TRANSFERASE (GGT). Policy Number: CMP-021.Effective Date: January 1, 2018.

87. Targher, G. (2010). Elevated serum gamma-glutamyltransferase activity is associated with increased risk of mortality, incident type 2 diabetes, cardiovascular events, chronic kidney disease and cancer a narrative review. Clin Chem Lab Me. 48: 147-157.

88. Eminler, A.T., Irak, K., Ayyildiz, T., Keskin, M., Kiyici, M., Gure, S., Gulten, M., Dolar, E., and Nak, S.G. (2014). The relation between liver histopathology and GGT levels in viral hepatitis: More important in hepatitis B. Turk J Gastroenterol; 25:411-5.

89. Traverso, N., Ricciarelli, R., Nitti, M., Marengo, B., Furfaro, A. L., Pronzato, M. A., and Domenicotti, C. (2013). Role of glutathione in cancer progression and chemoresistance. Oxidative medicine and cellular longevity, 2013.

90. Hosen, M. B., Karmokar, N. C., Karim, M. F., Al Mahmud, R., and Mesbah, M. (2015). Association of AST, ALT, ALB and Total Protein with Beta-thalassemia in Bangladeshi Population. International Journal, 3(1), 991-995.

91. Levitt, D. G., and Levitt, M. D. (2016). Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements. International journal of general medicine, 9, 229.

92. Nagao, Y., and Sata, M. (2010). Serum albumin and mortality risk in a hyperendemic area of HCV infection in Japan. Virology journal, 7(1), 375.

93. Saeed, H. S., Abdellah, A. M., Abdalla, F. A., Abbas, A. R. A., Adam, F. A., and ELgazali, N. A. (2017). Biochemical Effects of Lead Toxicity on Serum Total Protein, Albumin and Globulin Levels in Occupationally Exposed Workers in Major Sudanese Cities. International Journal of Emerging Technology and Advanced Engineering, 7(3), 132-138.

94. Pallister, C.J., and Watson, M.S. (2010). Haematology. Scion Publishing; pp: 336–347.

95. Kim, S., (2016). Fibrinogen. Healthline supports.MD on January 4, 2016 - Written by Corinna Underwood.

96. Sørensen, B., Tang, M., Hlarsen, O., Nlaursen, P., Fenger-Eriksen, C., and Jrea, C. (2011). The role of fibrinogen: a new paradigm in the treatment of coagulopathic bleeding. Thrombosis Research Volume 128, Supplement 1, 2011, Pages S13-S16.

97. Shao, Z., Zhao, Y., Feng, L., Feng, G., Zhang, J., and Zhang, J . (2015). Association between plasma fibrinogen levels and mortality in acute-on-chronic hepatitis B liver failure. Disease Markers. Volume 2015, Article ID 468596, 6 pages.

98. Lupien, G., Amess, C., Aubin, N., Baillargeon, L., and Lacroix, S. (2004). Factor 1 Deficiency Fibrinogen An inherited bleeding disorder. An information Booklet. Canadian Hemophilia Society.

99. Leticia, O.I., Andrew, A., Ifeanyi, O.E., Ifeoma, U.E., and Ugochukwu, A. (2014). The Effect of Viral Hepatitis ON APTT, PT, TT, Fibrinogen and Platelet among Blood Donors at FMC, Umuahia. IOSR Journal of Dental and Medical Sciences.Volume 13, Issue 8 Ver. V (Aug. 2014), PP 57-63.

100. Durackova, Z. (2010). Some current insights into oxidative stress.Physiological Research, 59(4), 459-469.

101. Ismail, H. K. (2017). The Effect of Catalase and Some Biochemical Parameters on Oxidative Stress in Acromegalic Patients. A Thesis Submitted to the College of Science AL-Mustansiriyah University in

Partial Fulfillment of the Requirements for the Degree of Master in Biochemistry.

102. Duygu, F., Karsen, H., Aksoy, N., Taskin, A. (2012). Relationship of oxidative stress in hepatitis B infection activity with HBV DNA and fibrosis. Ann Lab Med; 32(2):113-118.

103. Alavian, S.M., Showraki, A. (2016). Hepatitis B and Relationship with Oxidative Stress. Hepat Mon. 2016 Sep; 16(9): e37973.

104. Khajehnasiri, F., Mortazavi, S. B., Allameh, A., Akhondzadeh, S., and Hashemi, H. (2013). Total antioxidant capacity and malondialdehyde in depressive rotational shift workers. Journal of environmental and public health, 2013.

105. Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., et al (2018). Oxidative stress, aging, and diseases. Clinical Interventions in Aging 2018:13 757–772.

106. Lu, J., Wang, Z., Cao, J., Chen, Y., and Dong, Y. (2018). A novel and compact review on the role of oxidative stress in female reproduction. Reprod Biol Endocrinol. 2018 Aug 20; 16(1):80. doi: 10.1186/s12958-018-0391-5.

107. Pyari, J.S., Rekha, S., Singh, R.k., and Monica, A. (2006). Free radicals in female infertility. J. Obstet. Gynecol. India. 65(1): 64-67.

108. Mulla, A., Fazari, A.B.E., Elkhouly, M., and Moghaddam, N. (2018). Role of Antioxidants in Female Fertility. Open Journal of Obstetrics and Gynecology, 8, 85-91.

109. Al-Tai, R.A.A. (2013). Evaluating the Levels of Interleukin-33 (IL-33), Oxidative Stress, and Some Biochemical Parameters in Type 2

Diabetic and Nephropathic Patients. A Thesis Submitted to the Department of Chemistry, College of Education \ Ibn Alhaitham - University of Baghdad as Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry- Biochemistry.

110. Agarwa, A., Aponte-Mellado, A., Premkumar, B.J., Shaman, A., Gupta, S. (2012). The effects of oxidative stress on female reproduction: a review. Agarwal et al. Reproductive Biology and Endocrinology, 10:49.

111. Genestra, M. (2007). Oxyl radicals, redox-sensitive signaling cascades and antioxidants. Review. Cell Signal. 19(9): 1807-1819.

112. Koolman, J., and Roehm, K.H. (2005). Color Atlas of Biochemistry. 2nd ed. Thieme Stuttgart. New York. 77.

113. Andonova, L., Georgieva, M., and Zlatkov, A.l. (2015). Free radicals, oxidative stress and diseases associated with them PHARMACIA. 62: 26 – 39.

114. Elochukwu, C. (2015). Generation and reaction of free radicals in the human body: A major cause of aging and chronic degenerative diseases .EC Nutrition 1: 132-136.

115. Oyinloye, B. E., Adenowo, A. A., and Paul, K.A. (2015). Reactive oxygen species, apoptosis, antimicrobial peptides and human inflammatory diseases. Pharmaceuticals, 8:151-175.

116. Verma, A., Verma, A.K, and Baghel, S.S. (2015). Role of phytochemicals in neutralizing the adverse effects of ozone depletion. J. of Life Sciences, 3: 118-122.

117. Dewhirst, M. W., Cao, Y., and Moelle, B. (2008). Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. Macmillan Publishers Limited.8: 425- 437.

118. Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., and Kalayci, O. .(2012). Oxidative stress and antioxidant defense. World Allergy Organization, 5: 9–19.

119. Jan, A.T., Azam, M., Siddiqui, K., Ali, A., Choi. I., and Rizwanul Haq, Q. (2015). Heavy metals and human health: mechanistic insight into toxicity and counter defense system of antioxidants. Int. J. Mol. Sci, 16: 29592–29630.

120. Repetto, M., Semprine, J., and Boveris, A. (2012). Lipid peroxidation: chemical mechanism, biological implications and analytical determination. INTECH, 1:1-30.

121. Ayala, A., Muñoz, M. F., and Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondehyde and 4-hydroxy-2-nonenal. Hindawi Publishing Corporation, 2014:1-31.

122. Ismail, N. A., Okasha, S., Dhawan, A., Abdel Rahman, A. M. O, Abdel Hamid, N., and Shaker, O. (2012). Glutathione peroxidase, superoxide dismutase and catalase activities in children with chronic hepatitis .Advances in Bioscience and Biotechnology, 3: 972-977.

123. Jain, S., Nair, A., and Shrivastava, C. (2015). Evaluation of oxidative stress marker malondialdehyde level in the cord blood of newborn infants. International Journal of Scienti fi c Study, 3:73-76.

124. Rinnerthaler, M., Bischof, J., Streubel, M. K., Trost, A., and Richter, K. (2015). Oxidative stress in aging human skin. biomolecules journal, 5: 545-589.

125. Sharma, S.L., Chokshi, S.A., Desial, D., Mewada, H., and Singh, A. (2013). Non-enz ymatic antioxidants, malondehyde and total antioxidant activity as markes of oxidative stress in arthritis and rheumatoid arthritis. NHL Journal of medical sciences, 2:57-60.

126. Shohag, M.H., Ullah, M., Azad, M. A., Islam , M.S., Qusar, S., and Shahid, S. F.(2012). Serum antioxidant vitamins and malondialdehyde levels in patients with obsessive-compulsive disorder. Reprinted from the German Journal of Psychiatry, 1: 10-14.

127. Nagamani, M., Prahaladu, P., Vijayababu, P.V.S.S., Ashalata, K., kumari5, P. K. (2015). Lipid peroxidation product as a marker of oxidative stress in psoriasis -a case control study in north coastal. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), 5 :18-20.

128. Al-Aaraji, S.F.T.H. (2013). The Effect Of oxidative Stress In DNA Damage For Diabetic Mellitus Type Π Females. A Thesis Submitted to the Council of the College of Sciences, University of Anbar in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biochemistry.

129. Kattappagari, K.K., Teja, C.S.R., Kommalapati, R.K., Poosarla, C., Gontu, S.R., and Reddy, B.V.R. (2015). Role of antioxidants in facilitating the body functions: A review. J Orofac Sci, 7: 71-5.

130. Abe, M., Xie, W., Regan, M. M., King, I. B., Stampfer, M. J., Kantoff, P. W., and Chan, J. M. (2011). Single-nucleotide

polymorphisms within the antioxidant defence system and associations with aggressive prostate cancer. BJU international, 107(1), 126-134.

131. Matyas, M., Zaharie, G. (2019). Antioxidants at Newborns. University of Medicine and Pharmacy, Cluj-Napoca, Romania.© 2019 The Author(s). Licensee Intech Open.

132. Abedi, M., Mesbah-Namin, S.A., Noori-Zadeh, A., Tiraihi, T., and Taheri, T. (2018). Human wild-type superoxide dismutase 1 gene delivery to rat bone marrow stromal cells: its importance and potential future trends. Iran J Basic Med Sci, Vol. 21, No. 7, Jul.

133. Goodsell, D. S. (2007). Superoxide dismutase. RCSB PDB Molecule of the Month, 1-2.

134. Nedeljkovic, Z. S., Gokce, N., and Loscalzo, J. (2003). Mechanisms of oxidative stress and vascular dysfunction. Postgraduate medical journal, 79(930), 195-200.

135. Mohssan, S.N. (2018). Comparative study on Some Hormones, Trace Elements and Antioxidants levels in schizophrenia. A Thesis Submitted to the College of Science Mustansiriyah University in Partial Fulfillment of the Requirements for the Degree of Master of Science Chemistry (Biochemistry).

136. He, L., He, T., Farrar, S., Ji, L., Liu, T., and Ma, X. (2017). Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. Cellular Physiology an Biochemistry. 44(2): 532-553.

137. Sathler, P.C., Lourenço, A.L., Saito, M.S., Arêas, A.P.G., Rodrigues, C.R., Cabral, L.M., Helena, C., Castro, H.C, and Kang, H.C. (2016). THE ANTIHEMOSTATIC PROFILE OF VITAMIN C: MECHANISMS THAT UNDERLIE THE TECHNICAL APPLICATION OF A PHYSIOLOGICAL MOLECULE. Arch Biol Sci. 2016;68(2):325-331

138. Gupta, R.K., and Singh, N. (2013). Morinda citrifolia (Noni) alter oxidative stress marker and antioxidant activity in cervical cancer cell Lines. Asian Pac J Cancer Prev.14:4603-6.

139. Naji, T.S. (2016). Association between Oxidative Stress Status and (*CYP17, CYP19*) Genes Polymorphism in Endometriosis Iraqi Patients. A Thesis Submitted to the College of Science AL-Mustansiriyah University in Partial Fulfillment of the Requirements for the Degree of Doctorate of Philosophy in Biochemistry.

140. Michels, A., and Frei, B. (2012). "Vitamin C". In Caudill MA, Rogers M. Biochemical, Physiological, and Molecular Aspects of Human Nutrition (3 ed.). Philadelphia: Saunders: 627–654.

141. Mohammed, M.A. (2018). Synthesis and Characterization of Complex for Anti Breast Cancer, activity evaluation on Cell Line MCF-7 and Study of some Biochemical Parameters in Women with the disease.A Dissertation Submitted to the Council of College of Science University of Anbar in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biochemistry.

142. Doll, S., and Ricou, B. (2013). Severe vitamin C deficiency in a critically ill adult: a case report. Euro J of Clin. Nutr., 2013, 67, 881-882.

143. Al-Duleimi, H. SH. (2013). Study a number of antioxidants and some physiological change among women with breast cancer in Anbar Governorate. Thesis. College of Science / University of Anbar.

144. Pehlivan, F. E. (2017). Vitamin C: An Antioxidant Agent. In Vitamin C. Available at: http://dx.doi.org/10.5772/intechopen. Accessed:

145. Zimmermann, K.A. (2018). Immune System: Diseases, Disorders and Function. Live Science. ELSEVIER: 1–6.

146. Langley-Evans, S.C., Carrington, L.J (2006). Diet and the developing immune system. Lupus 15 (11): 746–52.

147. Byrne, K.J., Dalgleish, A.G. (2001). Chronic Immune Activation and Inflammation as the Cause of Malignancy. B.J.C. 85(4):473-483.

148. FERNANDES, J.V., FERNANDES, T.A.A., AZEVEDO, J.C.V., COBUCCI, R.N.O., CARVALHO, M.G.F., ANDRADE, V.S. and ARAÚJO, J.M.G. (2015). Link between chronic inflammation and human papillomavirus-induced carcinogenesis (Review). ONCOLOGY LETTERS 9: 1015-1026.

149. Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., and Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, Vol. 9, (No. 6), pp: 7204-7218.

150. Liu, M.M., Lei, X.Y.,Yu, H., Zhang, J.Z. and Yu, X.J. (2017). Correlation of cytokine level with the severity of severe fever with thrombocytopenia syndrome. Virology Journal, 14:6.

151. Ogawa, Y., and Calhoun, W.J. (2006). "The role of leukotrienes in airway inflammation". J.A.C.I. 118 (4): 789-98.

152. *Mannaa,* F.A*., and Abdel-Wahhab*, K.G. (2016). Physiological potential of cytokines and liver damages. Hepatoma Res; 2:131-43.

153. Khan, F. A., Al-Jameil, N., Khan, M. F., Al-Rashid, M., and Tabassum, H. (2015). Thyroid dysfunction: an autoimmune aspect. Review Article. Int J Clin Exp Med, 8(5):6677-6681.

154. Tawfig, N. (2016). Review Article: Proinflammatory Cytokines and Periodontal Disease. J Dent Probl Solut, 3(1): 012-017.

155. Alwan, A. H. (2015). Determination of Interleukin-1β (IL-1 β) and Interleukin-6(IL-6) in Gingival Crevicular Fluid in Patients with Chronic Periodontitis. IOSR- JDMS, 14 (11): 81-90.

156. Jarczak, J., Kaba, J., Reczy nska, D., and Bagnicka, E. (2016).Impaired Expression of Cytokines as a Result of Viral Infections with an Emphasis on Small Ruminant Lentivirus Infection in Goats. Viruses, 8 (186): 1-12.

157. Venkatesha, S. H., Dudics, S., Acharya, B., and Moudgil, K. D. (2015). Cytokine-Modulating Strategies and Newer Cytokine Targets for Arthritis Therapy. Int J Mol Sci, 16: 887-906.

158. Gulati, K., Guhathakurta, S., Joshi, J., Rai, N., and Ray, A. (2016). Cytokines and their Role in Health and Disease: A Brief Overview. MOJ Immunol 2016, 4(2): 00121

159. Mourtzikou, A., Alepaki, M., Stamouli, M., Pouliakis, A., Anastasios Skliris, A., and Karakitsos, P. (2014). Evaluation of serum levels of IL-6, TNF-, IL-10, IL-2 and IL-4 in patients with chronic hepatitis.I n m u n o l o g í a.; 33 (2):41–50.

160. Al-Tai, A.F.M. (2011). Study of Some Acute Phase Proteins, Interlenkin 8 And Oxidative Stress in Type II Iraqi Diabetic Patients. A Thesis Submitted to the Council of College of Education (Ibn-AL-Haitham) University of Baghdad in Partial Fulfillment of the Requirements for the Degree of Ph.D.In Biochemistry.

161. Turner, M.D., Nedjai, B., Hurst, T.,and Pennington, D.J. (2014). Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochimica et Biophysica Acta 1843, 2563–2582.

162. Hantoosh, S. F. (2017). Effect of Some Interleukins on Implantation after Intrauterine Insemination. A Dissertation Submitted to the Council of the College of Science AL-Mustansiriyah University in Partial Fulfillment of the Requirements for the Degree of Ph.D of Philosophy Science in Microbiology /Immunology.

163. Clements, A.E.B.,and Murphy, W.L. (2019). Injectable biomaterials for delivery of interleukin-1 receptor antagonist: Toward improving its therapeutic effect. Acta Biomaterialia 93, 123–134.

164. Abdulkhaliq, R.J. (2017). Role of Human Leukocyte Antigen- G and Cytokines in Recurrent Abortion. A Thesis Submitted to the council of College of Science - AL-Mustansiriyah University In Partial Fulfillment of the Requirements for the Degree of Doctor of philosophy in Biology/Zoology/ Immunology.

165. Lai, J., Liu, Y., Pan, C., Lin, C.,Sun, F., Huang, Z., Lin, Y., Zhou, R.,Lin, Y., and Zhou,Y. (2017). Interleukin-1 receptor antagonist expression is inversely associated with outcomes of hepatitis B-related acute-on-chronic liver failure.Experimental and therapeutic medicine 13; 2867-2875.

166. Oo, Y., Mutimer, D., and Adams, D. (2012). Low-dose interleukin-2 and HCV-induced vasculitis. N Engl J Med. 366:1353-1354.

167. Wrenshall, L., Clabaugh, S., Cool, D., Arumugam, P., Grunwald, W., Smith, D., Liu, G., and Miller, J. (2014). Identification of a cytotoxic form of dimeric interleukin-2 in murine tissues. PLoS One. **9**:e102191.

168. Al-Saffar, O.B. (2015). The Predicative Significance of Some Cytokine Genetic Polymorphisms in Iraqi Patients with Hepatitis B and C Viral Infections. A Thesis Submitted to the College of Science/ University of Baghdad in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Microbiology/Immunogenetics.

169. Waldmann, T. (2006). The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. Nat Rev Immunol., 6:595-601.

170. Rajasagi, N.K., Rouse, B.T. (2016). IL-2 complex treatment amplifies CD8 T cell mediated immunity following herpes simplex virus-1 infection. Microbes Infect.2016 Dec; 18(12): 735-746.

171. Castillo, F.M., Romero, T.A., ´Vez, J.E., Costa, L.L., Atencio, R., Porto, L., and Callejas, D. (2002). Concentrations of Cytokines, Soluble Interleukin-2 Receptor, and Soluble CD30 in Sera of Patients

with Hepatitis B Virus Infection during Acute and Convalescent Phases. Clin Diagn Lab Immunol. Nov; 9(6) 1372–1375.

172. Al-Kenzawi N.A.H.H. (2016). Study of Herd Immunity Associated with Hepatitis B Virus Infection in Basra Province South of Iraq. Thesis Submitted to the Council of College of Science-University of Basra In partial fulfillment of the requirements for The Degree of MSc. in Molecular Immunity.

173. Ilan, Y. (2002). Immune downregulation leads to upregulation of an antiviral response: a lesson from the hepatitis B virus. J. Microb Infect. 4:1317-1326.

174. Jung, M.C., and Pape, G.R. (2002). Immunology of hepatitis B infection. Lancet Infect Dis. 2:43-50.

175. Rapicetta, M., Ferrari, C., and Levrero, M. (2002). Viral determinants and host immune responses in the pathogenesis of HBV infection. J. Med Virol. 67:454-457.

176. Bozkaya, H., Bozdayi, M., and Turkyilmaz, R. (2000). Circulating IL-2, IL-10 and TNF-α in chronic hepatitis B: their relations to HBeAg status and the activity of liver disease. J. Hepato. gastroenterol. 47:1675-1679.

177. Xia, Y., and Protzer, U. (2017). Control of Hepatitis B Virus by Cytokines. Viruses 2017, *9*, 18; doi: 10.3390/v9010018.

178. Abushady, E.A., Gameel, M.M.A., Klena, J.D., Ahmed, S.F., Abdel-Wahab, K.S.E., and Fahmy, S.M. (2011). HBV vaccine efficacy and detection and genotyping of vaccineé asymptomatic breakthrough HBV infection in Egypt. J. Hepatology. 3(6): 147-156.

179. Kesieme, E.B., Uwakwe, K., Irekpita, E., Dongo, A., Bwala, K.J., and Alegbeleye B.J. (2011). Knowledge of Hepatitis B Vaccine among Operating Room Personnel in Nigeria and Their Vaccination Status. J. Hindawi Publishing Corporation. 2011:5pages.

180. Soyemi, K., Pendergrass, K., and Freeman, D. (2012). Perinatal Hepatitis B Infant Post-Vaccination Serologic Testing. Illinois Department of Public Health. 525-535.

181. Al-Ouqaili, M.T.S. (2018). Molecular Detection of Insertion Sequence 6110 of Mycobacterium Tuberculosis in Patients with Pulmonary Tuberculosis and Tuberculous Pleuritis in Anbar Governorate, West of Iraq. Int J Life Sci. Pharm Res. 2018. 8(3): 146-57. DOI:10.22376/ijpbs/lpr.2018.8.3.l46-57.

182. Kang, Q., Parkin, B., Giraldez, M. D., and Tewari, M. (2016). Mutant DNA quantification by digital PCR can be confounded by heating during DNA fragmentation. BioTechniques, 60(4), 175–185.

183. Khan, F., Shams, S., Qureshi, I.D., Israr, M., Khan, H., Sarwar, M.T., and Ilyas, M. (2011). Hepatitis B virus infection among different sex and age groups in Pakistani Punjab. J. Virology. 8:225-229.

184. Boom, R., Sol, C.J., Heijtink, R., Wertheim-van Dillen, P.M., and Noordaa, J. (1991). Rapid purification of hepatitis B virus DNA from serum. J. Clinical Microbiology. 29:1804–1811.

185. Green, M.R., and Sambrook, J. (2012) Molecular Cloning: A Laboratory Manual. Vol. 3. Cold Spring Harbor, New York: Set Cold Spring Harbor Laboratory Press;2012.

186. AL-Ani, S.K., AL-Ouqaili, M.T.S., and Awad, M.M. (2019). THE ROLE OF OXIDATIVE STRESS MARKERS IN B-THALASSEMIC IRAQI PATIENTS INFECTED WITH HEPATITIS C VIRUS DIAGNOSED BY RT-PCR. Int. J. Life Sci. Pharma Res. 2019; 9(1): (L) 13-23

187. Al-Ani, S.K.A. (2019). Prevalence of SEN Virus in Thalassemic Infected Patients with Hepatitis C Virus: Molecular and Physiological Study. A Dissertation Submitted to the Council of College of Science- University of Anbar in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biology / Microbiology.

188. Shaban, N.Z., Salem, H.H., Elsadany, M.A., Ali, B.A., Hassona, E.M., Mogahed, F.A. (2014). Alteration in Lipid peroxidation and Antioxidant in patients with Different Stage of Hepatitis B Virus Infection in Egypt Life Science Journal. 2014. 11(8).

189. Bijedic, Z.V., Mehmedovic, A., Redzepovic, A., Gogov, B. (2014). Use of serum levels of proinflammatory cytokine IL-1α in chronic hepatitis B. Med Arh. 2014. 68(2): 94-97.

190. Daniel, W.W., and CROSS, C.L. (2013). Biostatistics, A Foundation For analysis in the health sciences ., John Wiley and sons. New York, p: 958.

191. Thageel, R.Q. (2016). Role of Interferon lambda and Toll like receptor- 2 in hepatitis B virus infection. A thesis Submitted to the College of Science/ University of Anbar as a Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology /Microbiology.

192. Al-Suraifi, A.S.K. (2016). Molecular and serological study of hepatitis B virus genotypes in Wasit province\Iraq. A Thesis Submitted to the council of the college of science Wasit University in partial fulfillment of the requirement for the degree of Master of Science in Biology (Zoology).

193. Issa, A. H., Hamdan, N. A., and Thwiny, H. T. (2015). Hepatitis B Virus Subclinical Case Infection In Basra Patients Discovered By Pre Operation of Viral Screening Investigations. World Journal of Pharmaceutical Research.4 (4):1599-1610.

194. Al-Hilli, H. A., and Ghadhban, J. M. (2000). Prevalence of serological markers of HBsAg and HCV-antibodies among blood donors and certain risk groups. Journal of Faculty Medicine. Baghdad.42(1):45-49.

195. Al-Waysi, S. A. (2005). Effectiveness of interferon-alfa and lamivudine drugs in the treatment of chronic viral hepatitis (B and C) among Iraqi patients. Ph.D. Thesis, College of Medicine, University of Baghdad.

196. Al-Azzawi, R. H. M. (2007). A Comparative Study of Some Immunological Aspects In Vivo and In Vitro in Patients with Chronic Hepatitis B And C Virus (Pre-and Post-Treatment). Ph.D. Thesis, College of Science, University of Baghdad.

197. Sanchez-Tapias, J. M., Costa, J., Mas, A., Bruguera, M., and Rodés J. (2002). Influence of HBV genotype on the long term outcome of chronic HBV in western patients. Journal of Gastroenterology.123 (6):1848-1856.

198. Wasley, A., Kruszon-Moran, D., Kuhnert, W., Simard, E. P., Finelli, L., and McQuillan, G. *et al*. (2010). The Prevalence of Hepatitis B Virus Infection in the United States in the Era of Vaccination. The Journal of Infectious Diseases.202(2):192-201.

199. Fang, Y., Shang, Q. L., Liu, J. Y., and Li, D. (2009). Prevalence of occult hepatitis B virus infection among hepatopathy patients and healthy people in china. Journal of Infectious disease.58:383-588.

200. Jaroszewicz, J., Serrano, C. B., Wursthorn, K., Schlue, J., Raupach, R., Flisiak, R. *et al*. (2010). Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV) infection- a European perspective. Journal of Hepatology.52 (4):514-522.

201. Brunetto, M.R. (2010). A new role for an old marker, HBsAg. J Hepatol. 52:475-7.

202. Zhang, Y.M., Yang, Y.D., Jia, H.Y., Zeng, L.Y., Yu, W., Zhou, N., and Li, L.J. (2014). HBsAg levels in HBeAg-positive chronic hepatitis B patients with different immune conditions. World J Gastroenterol. 2014;20:4407-13. doi: 10.3748/wjg.v20.i15.4407.

203. Kao, J.H. (2008). Diagnosis of hepatitis B virus infection through serological and virological markers. Expert Rev Gastroenterol Hepatol. 2008;2:553-62. doi: 10.1586/17474124.2.4.553.

204. De Oliveira, V.O.B., Oliveira, J.P.R., De França, E.V.C., de Farias Brito ,H L., Nascimento, T.V., and França, A. (2016). Advanced liver injury in patients with chronic hepatitis B and viral load below 2,000 IU/mL. Rev Inst Med Trop Sao Paulo. 2016:58-65. doi: 10.1590/S1678-9946201658065.

205. Nita, M.E., Gaburo, N.J., Cheinquer, H., L'Italien, G., Araujo E.S., Mantilla, P., Cure-Bolt, N., and Lotufo, P.A. (2009). Patterns of viral load in chronic hepatitis B patints in Brazil and their association with ALT levels and HBeAg status. J. ANNALS of Hepatology. 8(4):339-345.

206. Al-khozai, Z.M. (2006). Study Some Epidemiological and Immunological aspects on Viral Hepatitis type B and C in Al-Qadisiyia Province. Ph. D Thesis. college of Science. University of Baghdad.

207. Zaidan, T.A., alfaluji, A.W., and Saod, W.M. (2016). Renal Function Status of Fallujah Patients (IRAQ) Infected with Chronic Hepatitis B. Journal of Babylon University/Pure and Applied Sciences/ No. (7)/ Vol. (24).

208. Ormeci, A., Aydın, Y., Sumnu, A., Baran, B., Soyer, O. M., Pınarbasi, B., et al. (2016). Predictors of treatment requirement in HBeAg-negative chronic hepatitis B patients with persistently normal alanine aminotransferase and high serum HBV DNA levels. International Journal of Infectious Diseases 52: 68–73. ELSEVIER.

209. Wang, X.H., Cheng, P.P., Jiang, F., and Jiao, X.Y. (2013). The effect of hepatitis B virus infection on hepcidin expression in hepatitis B patients. Ann Clin Lab Sci. Spring; 43(2):126-34.

210. Önlen, Y., Savaş, L., Özer, B., and İris, N.E. (2006). The Elevation of Liver Enzymes Due to Hepatitis B Vaccine. Eur J Gen Med 2006; 3(4):197-200

211. Park, W.Y., Koh, E.S., Kim, S.H., Kim, Y.O., Jin, D.C., Song, H.C., et al. (2015). Serum GammaGlutamyltransferase Levels

Predict Clinical Outcomes in Hemodialysis Patients. PLoS One. 2015; 10(9): e0138159. DOI:10.1371 /journal. pone.0138159.

212. Fiorino, S., Conti, F., Gramenzi, A., Loggi, E., Cursaro, C., Di Donato, R., Micco, L., Gitto, S., Cuppini, A., Bernardi, M., and Andreone, P. (2011). Vitamins in the treatment of chronic viral hepatitis. Br J Nutr. 2011;105:982–9.

213. Fialkow, L., Wang, Y., and Downey, G.P. (2007). Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. Free Radic Biol Med. 2007; 42:153-64.

214. Moulasl, A.N., Noulas, A., Papadamou, M.G., Bonanou-Tzedaki, S., and Dalekos, G.N. (2004). Total antioxidant status and erythrocyte superoxide dismutase activity in patients with chronic hepatitis B and C. European Journal of Inflammation. 2004;2:77-84.

215. Dakrory, A.I., Al Harbi, M.S., and Mohamed, A.S. (2014). Antioxidant role of holothuria atra extract against nephrotoxicity induced by 7,12-dimethylbenz (a) anthracene in male albino rats. International Journal of Advanced Research. 2014;3:275-87.

216. Kökoğlu, O.F., Uçmak, H., Toprak, R., Kuzhan, N., Kurutaş, E.B., Atalay, F., Çetinkaya, A., and Kantarçeken, B. (2012). Oxidative stress biomarkers in urine of patients with hepatitis B and C. Balkan Medical Journal. 2012;29:39-42.

217. Ming, D.S., Lin, Z.Z., and Qiu, X.D. (2016). Analysis of IL-6, IL-10, IL-17 levels in the blood of patients with chronic hepatitis B. International Journal of Clinical and Experimental Medicine. 2016;9:9437-42.

218. Fujinami, R.S., Oldstone, M.A. (1979). Antiviral antibody reacting on the plasma membrane alters measles virus expression inside the cell. Nature. 1979;279:529–30.

219. Maini, M., Schurich, A. (2010). The molecular basis of the failed immune response in chronic HBV: Therapeutic implications. J Hepato.l 2010; 52:616-9.

220. Franceschi, C., Capri, M., Monti, D., Caruso, C., Candore, G., Vasto, S., Oliveri, F., Marchegiani, F., Sansoni, P., Baggio, G., Mari, D., Passarino, G., and DeBenedictis, G. (2007). Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev., 128:92-105.

221. Zhu, S., Patel, K. V., Bandinelli, S., and Ferrucci, G. J. (2009). Predictors of interleukin-6 elevation in older adults. J Am Geriatr Soc., 57:1672-1677.

222. Goetzl, E., Huang, M., Kon, J., Patel, K., Schwartz, J., Fast, K., Ferrucci, L., Madara, K., Taub, D. and Longo, D. (2010). Gender specificity of altered human immune cytokine profiles in aging. FASEB J., 24:3580-3589.

223. Elahe, T., Crockett, W., Spielman, S., Dowlatshahi, A., and Jun, H. (2006). Sex differences in inflammatory cytokine production in hepatic ischemia-reperfusion. J Inflamm., 3:16-22.

224. Klingstrom, J., Lindgren, T., and Ahlm, C. (2008). Sex-Dependent Differences in Plasma Cytokine Responses to Hantavirus Infection. Clin Vaccine Immunol., 15:885-887.

225. Poveshchenko, A., Orlov, N., Kazakov, O., Poveshchenko, O. Kim, I., Bondarenko, N., Miller, T., Strunkin, D., Kabakov, A., Reiter, T., and Konenkov, V. (2014). Age and Gender Differences in Cytokine Profile of Lymph and Blood Serum. Advances in Aging Research, 3:216-221.

226. Helal, E.G., El-Sayed, A.F.M., Abu- Ouf, N., Mohamed, N.G., and Ahmed, M.A.M. (2013). Hematological and Immunological Studies on the Effect of Hepatitis B Virus Vaccination in Hepatitis and Non-Hepatitis, Iron Chelating Dependent or Independent Egyptian Thalassemia Patients. The Egyptian Journal of Hospital Medicine. Vol. 53, Page 1049– 1063.

227. Hayakawa, M. (2017). Dynamics of fibrinogen in acute phases of trauma. Hayakawa Journal of Intensive Care, 5:3. DOI 10.1186/s40560-016-0199-3.

228. Rathod, N.N., Nair, S.C., Mammen, J., and Singh, S. A. (2016). Comparison study of routine coagulation screening tests (PT and APTT) by three automated coagulation analyzers. International Journal of Medical Science and Public Health | 2016 | Vol 5 | Issue 08.

229. William, F.G., (2003). Hemostasis. In the textbook of review of Medical Physiology. 21st Edition.McGraw-Hill. p. 543-546.

230. Mishra, N.R., Panda, P.C., and Das, S. (2013). PROTHROMBIN TIME & INTERNATIONAL NORMALIZED RATIO IN PEDIATRIC LIVER DISEASE. Journal of Global Biosciences Vol. 2(6), pp. 241-246.

231. Balkan, A., Namıduru, M., Balkan, Y., Mete, A.Ö., Karaoğlan, I., and Boşnak, V.K. (2016). Are Serum Quantitative Hepatitis B Surface Antigen Levels, Liver Histopathology and Viral Loads Related in Chronic Hepatitis B-infected Patients? Saudi J Gastroenterol. 2016 May-Jun; 22(3): 208–214.

232. Harrison, M.F. (2018). The Misunderstood Coagulopathy of Liver Disease: A Review for the Acute Setting. Western Journal of Emergency Medicine. Volume 19, no. 5: September 2018.

233. Goldsby, G., Kindt, B., and Obsorne, N. Kuby Immunology. (2007). 6th edition.W.H. Freeman Company.

234. Ifeanyi, O.E., Onyenweaku, F.C., Nwobodo, H.A., Ochei, K.C., Ochiabuto Ogochukwu, M.T.B., Francisca, O.U., Jacinta, N.C., (2017). Chukwuebuka UB. Impact of HIV and, hepatitis B Virus coinfection on Selected Haematological Markers of the patients in Umuahia, Abia State, Nigeria. Annals of Clinical and Laboratory Research. Vol.5 No.2:175. ISSN 2386-5180.

235. Qamar, A. A., and Grace, N.D. (2009). Abnormal hematological indices in cirrhosis.Can J Gastroenterol; 23(6):441-445.

236. Pan, Y., Muheremu, A., Wu, X., and Liu, J. (2016). Relationship between platelet parameters and hepatic pathology in patients with chronic hepatitis B infection – a retrospective cohort study of 677 patients. Journal of International Medical Research. Vol. 44(4) 779–786.

237. AlKINANI, A.M.S. (2014). Hematological and Biochemical Study of Hepatitis B and C Patients in Wasit Province. Wasit University in

Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology.

238. AL-Khilkhali, H.J.B. (2017). Determination of some hematological parameters in hepatitis B patients in Najaf city. Journal of Babylon University/Pure and Applied Sciences/No.(3)/Vol.(25).

239. Francisca, O.U., Ihongbe, J.C., Ifeanyi, O.E., Martin, O. I., Ebele, N.P, and Ochiabuto Ogochukwu, M.T.B. (2017). Evaluation of Some Immunological and Haematological Indices of Hepatitis B Infected Subjects in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. Journal of Biomedical Sciences. Vol. 6 No. 3:17 ISSN 2254-609X.

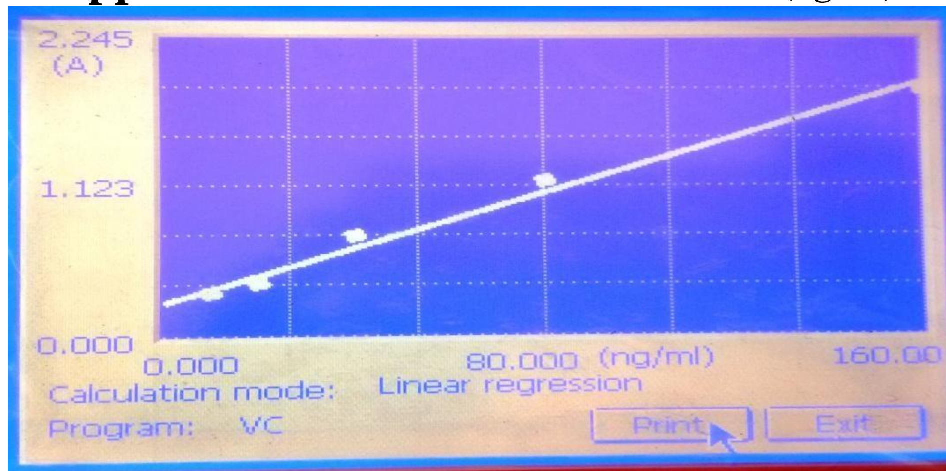
240. Fasola, F.A, Otegbayo, J.A, Abjah. U.M.A, and Ola, S.O. (2009). Hematological PARAMETERS IN NIGERIANS WITH ACUTE VIRAL HEPATITIS. Nigerian Journal of Gastroenterology and Hepatology Vol.1 No1 June 2009: 27-31.

Appendixes

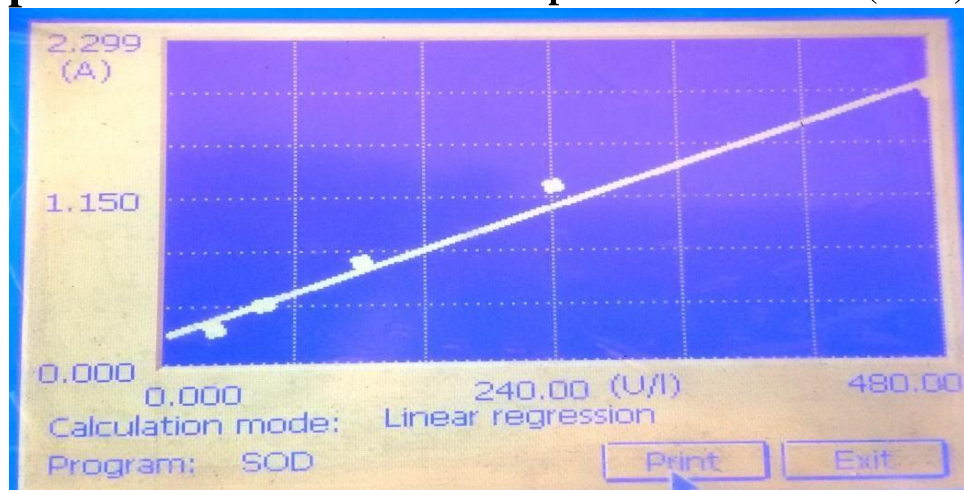
Appendix-1
Questionnaire sheet

INFORMATION	DETAILS
Case No.	
Date	----- /---- / ----
Name	
Age	
Gender	Female Male
Address	
Clinical Status	
Drug history	
Other notes	
Immunodipstik assay	
TOSOH Analyzer	
RT-PCR	
ELISA	
Liver function tests	ALT(----), AST(----), ALK(---),TSB(---),TP(----)
Oxidative stress parameters	Vitamin C SOD MDA
Cytokines parameters	IL-1alpha IL-2
Hematological tests	WBC(---), RBC(---),Hb(---),PLT(---)

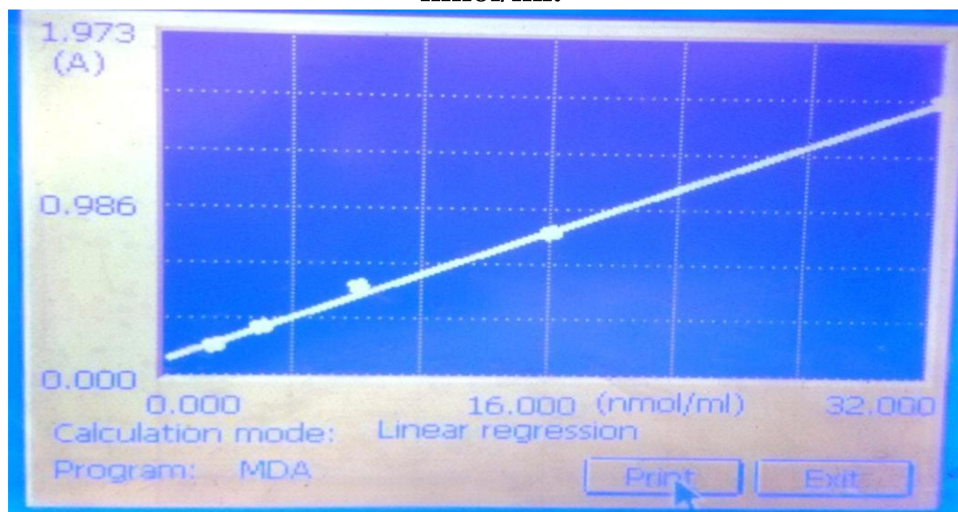
Appendix-2/ Standard curve of vitamin C (ng/ml).



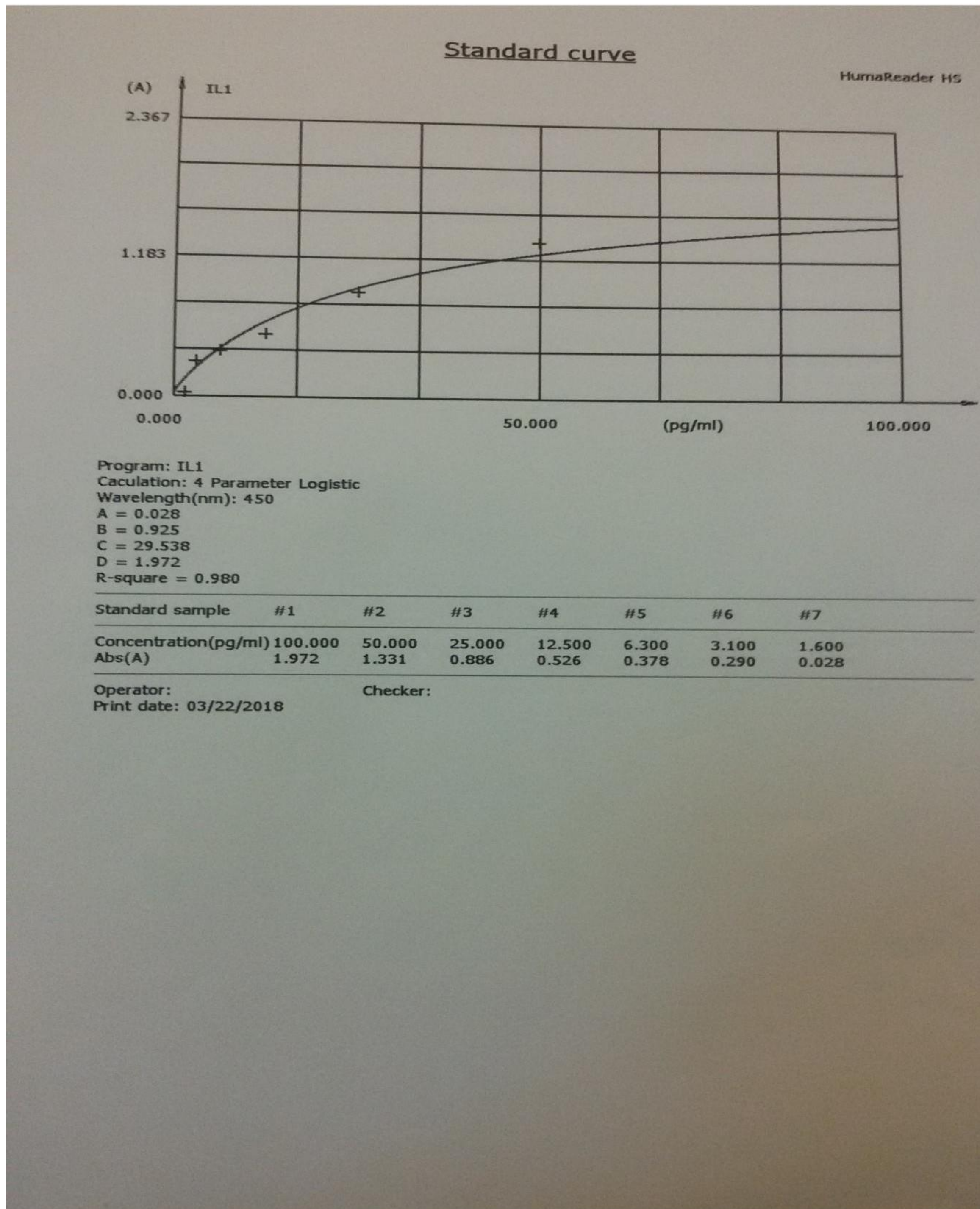
Appendix-3/ Standard curve of Superoxide dismutase (SOD) U/l.



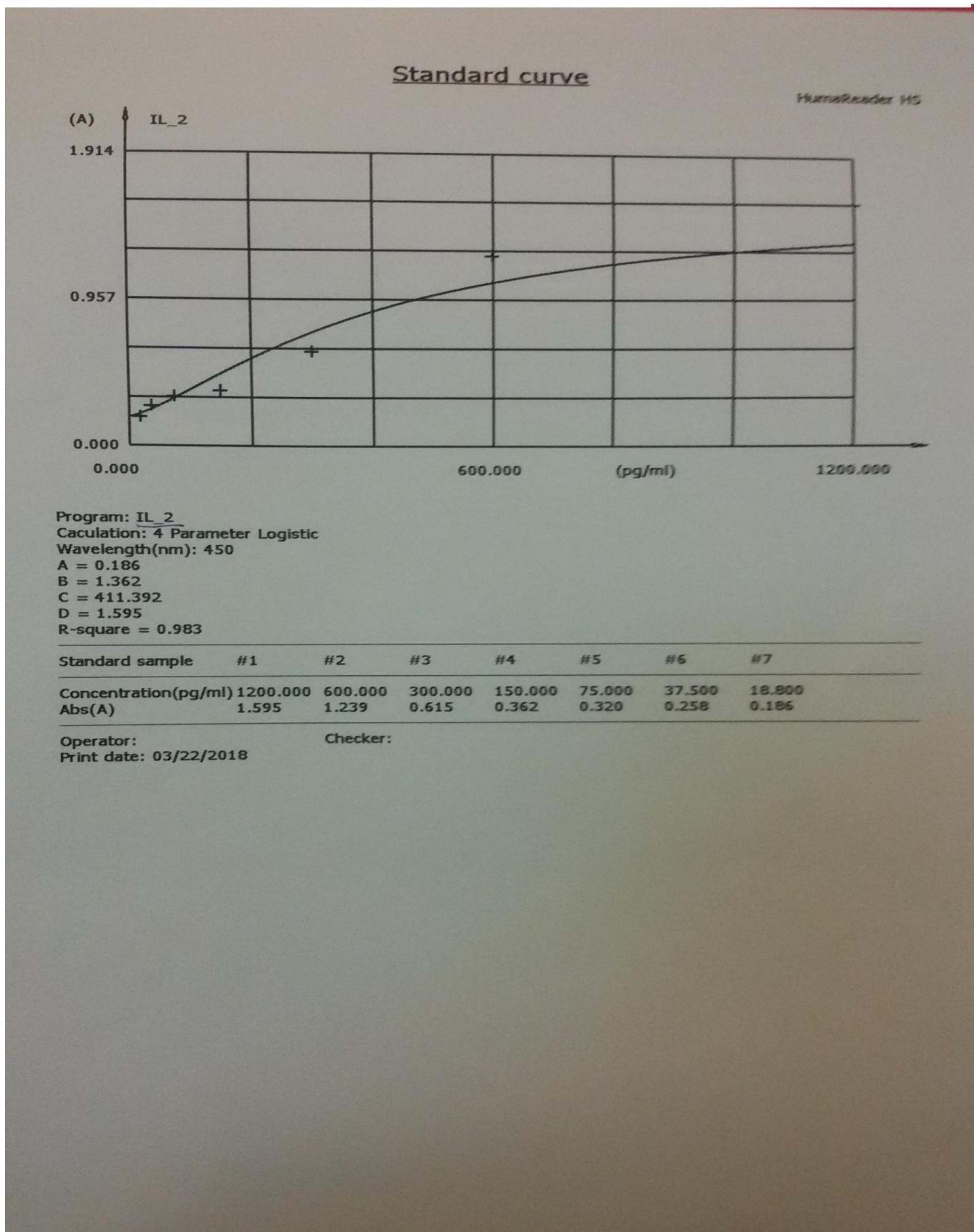
Appendix-4/ Standard curve of Malondialdehyde (MDA) nmol/ml.



Appendix-5/ Standard curve of Interlukin-1 alpha (pg/ml).



Appendix-6/ Standard curve of Interlukin-2 (pg/ml).

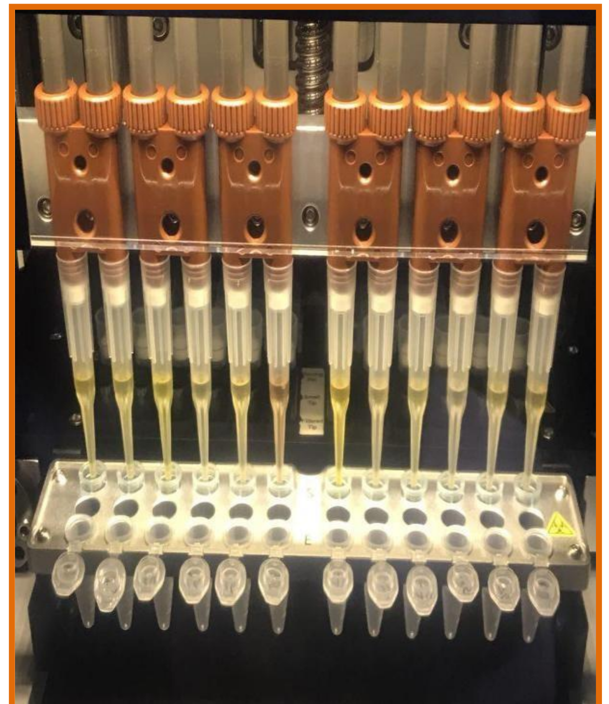


Program: IL_2
Caculation: 4 Parameter Logistic
Wavelength(nm): 450
A = 0.186
B = 1.362
C = 411.392
D = 1.595
R-square = 0.983

Standard sample	#1	#2	#3	#4	#5	#6	#7
Concentration(pg/ml)	1200.000	600.000	300.000	150.000	75.000	37.500	18.800
Abs(A)	1.595	1.239	0.615	0.362	0.320	0.258	0.186

Operator: Checker:
Print date: 03/22/2018

Celercare® M1 Automatic Chemistry Analyzer used in this study



SaMag-12/24 Automatic Nucleic Acids Extraction System used in this study.

Smart Cycler Real-time PCR instrument used in this study.

Automated chemistry analyzer TOSOH, AIA-1800 ST used in this study.

الخالصة

التهاب الكبد نمط)ب(هو عدوى تهدد الحياة وتسببها انواع غير محددة من الفيروسات تسمى فيروس التهاب الكبد نمط)ب(. العراق من بين البلدان المتوسطة المتوطنة لفيروس االلتهاب الكبدي الوبائي البائي ألن معدل اإلصابة بفيروس التهاب الكبد الوبائي البائي يتراوح بين)3٪ -5.4٪(بين السكان و)2٪ -3٪(بين المتبرعين بالدم الصحيين على ما يبدو ، وبالتاليتم تصميم هذه الدراسة. اشتملت الدراسة على 54 مريض في الطور المعدي لمرض التهاب الكبد الفيروسي نمط)ب(HBeAg) موجب(مقسم الى 24 انثى و 24 ذكرا ، و 54 مريض في الطور غير المعدي لمرض التهاب الكبد الفيروسي نمط)ب(HBeAg) سالب(مقسم الى 81 انثى و22 ذكرا، و 54 من االفراد االصحاء مقسم الى 81 انثى و 28 ذكرا التي تضمنتها هذه الدراسة خالل الفترة من شباط ولغاية ايلول لعام 2481. أجريت الدراسة في مستشفى العامرية العام / محافظة االنبار.

أجري االختبار المناعي السريع للكشف عن االجسام المضادة لفيروس التهاب الكبد نمط)ب(في مصل المرضى، وتم استخدام جهاز المحلل الكيميائي االوتوماتيكي -TOSOH, AIA 1800 ST)(لتشخيص مستوى HBsAg كأختبار تأكيدي. أجريت الفحوصات الكيموحيوية لتقدير فعالية االنزيمات {االنزيم الناقل لمجموعة االمين لحامض االنيين)ALT) ، االنزيم الناقل لمجموعة االمين لحامض االسبارتين (AST)، انزيم الفوسفتيز القاعدي (ALP) ،وكذلك البليروبين الكلي (TSB) ، وكاما كلوتاميل ترانسفيرز)GGT(والبروتين الكلي:} األلبومين (ALB(والكلوبيولين (GLO) } باستعمال جهاز المحلل الكيميائي األوتوماتيكي Automatic Chemistry Analyzer Instrument (MNCHIP) لكل عينة مصل للمرضى وعينات مجموعة السيطرة فضال عن تقدير مستوى مضادات األكسدة غير األنزيمية التي تشمل)فيتامين C(واالنزيمية سوبر اوكسايد دسميوتيز)SOD(باألضافة الى تقدير مستوى انترلوكين الفا واحد)IL-1 alpha(وانترلوكين 2 (IL-2(باستخدام تقنية المقايسة المناعية المرتبطة باالنزيم (ELISA).

تم استخالص الحمض النووي ال)HBV- DNA) باستعمال جهاز إستخالص الحمض النووي األوتوماتيكي)SaMag-12(. بعدها تم كشف وتقدير معيار الحمض النووي الرايبي لفيروس الكبد نمط)ب(في البالزما باختبار البلمرة المتسلسل الكمي. اظهرت النتائج ان كل المرضى المصابين بالتهاب الكبد الفيروسي نمط)ب( )14مريض(كانت نتيجتهم موجبة ل

HBsAg. حيث ان مستوى HBsAg يزداد في امصال مرضى HBeAg موجب مقارنة بمرضى HBeAg سالب (7779.9 ± 3898 ,3233.8 ± 2474) وحدة دولية / مل على التوالي. فيما يتعلق باختبار معيار الفايروس، كانت كمية الفيروس (Viral load)، من الأجسام المضادة الإيجابية لـ HBV بزيادة لمرضى HBeAg موجب مقارنة بمرضى HBeAg سالب (35328825 ± 23101537 ، 3115.1 ± 1916.8(وحدة دولية / مل.

اجري الاختبار المناعي السريع لكل عينات المرضى التي كانت نتيجة HBsAg لهم موجبة لتشخيص: HBsAg، HBsAb، HBeAg، HBeAb، و HBcAb. في الطور المعدي لمرض التهاب الكبد الفيروسي نمط)ب(للمرضى الذين كان لديهم HBeAg موجب، وجد ان Anti- HBsAg لم يشخص في أي من المرضى. وكذلك وجد ان كل المرضى بنسبة)844%(كان لديهم Anti-HBs سالب، بالاضافة الى ذلك وجد ان ال Anti-HBeAg بنسبة 2.4 % من المرضى بينما وجد ان ما يعادل 4.ـ1 % من المرضى كان لديهم Anti-HBeAg سالب. كذلك وجد ان Anti-HBc تم تشخيصه بنسبة 4.ـ5 % من المرضى.

أما فيما يتعلق بالجانب الكيموحيوي والفسيولوجي فقد أظهر التحليل الإحصائي أن هناك زيادة بشكل ملحوظ في متوسط مستوى AST، ALT، ALP في مرضى HBeAg الموجب مقارنه بمرضى HBeAg السالب والاشخاص الاصحاء (P = 0.001). أما بخصوص مستوى GGT وجد ان هناك زيادة ملحوظه لمرضى HBeAg الموجب ومرضى HBeAg السالب مقارنة بالاشخاص الاصحاء (P value=0.002). في حين لم يكن هناك تغير معنوي في كل من مستويات البليروبين الكلي ،البليروبين المباشر وغير المباشر، البروتين الكلي، الالبومين والكلوبيولين. اما بالنسبة لمؤشرات الاجهاد التأكسدي، وجد ان هناك انخفاض في معدل فيتامين سي)V.C(لمرضى HBeAg الموجب والمتطوعين الاصحاء. ومع ذلك وجد ان هناك انخفاض معنوي في مستوى المالوندايالديهايد)MDA(و سوبر اوكسايد دسميوتيز)SOD(لكل من مرضى HBeAg الموجب والسالب مقارنه بما كان عليه للمتطوعين الاصحاء.

وفيما يتعلق بالجزء المناعي، ظهرت معدالت IL-1 alpha بمستويات متشابهه في المجموعات الثالثة من الافراد. ولم تظهر مستويات IL-2 اختالفا معنويا بين مرضى HBeAg الموجب والافراد الاصحاء بينما وجد ارتفاع معنوي بمستوى IL-2 لمرضى HBeAg السالب مقارنة بمرضى HBeAgالموجب ومجموعة السيطرة كالهما (P=0.001).

توصلت الدراسة الى ان تفاعل البلمرة المتسلسل هو افضل تقنية لتشخيص وجود ومراقبة نسخ DNA HBV- وان المؤشرات الكيموحيوية خاصة ALT، AST، GGT، ALP كانت مرتفعة لمرضى التهاب الكبد الفيروسي بي بشكل ملحوظ. وان فعالية فيتامين C كانت منخفضة لمرضى HBeAg الموجب بينما فعالية SOD كانت منخفضة لكل من مرضى HBeAg الموجب والسالب مقارنة باالصحاء. وان فعالية MDA انخفضت بشكل ملحوظ لمرضى التهاب الكبد الفيروسي بي. باالضافة الى ذلك وجود انخفاض في مستوى IL-2 لمرضى HBeAg الموجب نسبة الى مرضى HBeAg السالب.

جمهورية العراق و
زارة التعليم العالي والبحث العلمي
جامعة االنبار قسم – كلية العلوم
الكيمياء

دراسة المؤشرات الجزيئية و الكيموحيوية لمرضى التهاب الكبد الفيروسي نمط B المزمن في محافظة االنبار

اطروحة

مقدمة إلى مجلس كلية العلوم في جامعة ألنبار

كجزء من متطلبات نيل درجة الدكتوراه فلسفة في

علوم الكيمياء

من قبل

بان محمد عباس كنعان التميمي

بكالوريوس علوم كيمياء – جامعة االنبار)0222(

ماجستير علوم كيمياء – جامعة االنبار)0202(

بإشراف

أ. د. مشتاق طالب صالح العكيلي **أ.م. د. خالد فاروق عبد الغفور الراوي**

1441 هـ **0220 م**