

قال الله تعالى

وَقُلْ أَعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ۖ وَلَا يَرَوْنَ  
إِلَّا ۖ عَالَمٌ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ

صدق الله العظيم

سورة التوبة الآية 105

## **Dedications**

I dedicate this research to my parents

To my husband the persons whom I love, respect and

appreciated.

.....

My brothers and sisters

For their support and kindness

My Friends and Colleagues.

## **Acknowledgment**

This research took me almost a seven month, by that time; I have met with great people whose contribute in many ways came out with this project. It is a pleasure to convey my gratitude to them all in my humble acknowledgment.

Firstly I would like to note my gratitude to Dr. Hassan Elsiddig Hassan for his supervision, advice, encouragement and guidance from the very early stage of this research as well as attending meetings and conferences. I am in debt to him more than his knows.

Individual acknowledgment to my husband Mr. Esam Abdalbasit M. Alhassan for his advice and bright thoughts which came during scientific discussions and debates which were very fruitful for shaping up my ideas and research.

I gratefully and collective acknowledgment the role of my colleagues in the Central Laboratory Dr. Khalid Enan, Miss. Dalia Murse, Mr. Abdulhaffez Abdulazim, Miss. Yusra Abdulmalik, Dr. Abdulgaffar fahal and Mr. osama M Alkher for their encouragement and their bright thoughts which came during scientific discussion's, also for their willingness in answering a lot of my questions, advice and crucial contribution.

Thanks for the scientific discussions and exhilarating time we spent together as lab mates. I am grateful in every possible way.

My deep thanks are extended to Miss. Nada Saleh for her advice and support me. Also staff members of Dr. Fedil and IbnSina Hospitals for their help and support me in collection of specimen. My thanks go to them for working together in such environment.

Finally, I would like to thanks everybody who was important to the successful realization of this research, as well as expressing my apology to those who I could not mention personally one by one.

Sara O.A. Boshara

## Abstract

This is a descriptive retrospective study carried out in central laboratory-Sudan during the period between February 2012 to July 2012. The study aimed at detection of the association of Epstein-Barr virus with oesophageal and stomach cancers among Sudanese Patients using Polymerase Chain Reaction. Samples from 39 patients were included (21 with stomach cancer and 18 with esophagus cancer) their ages ranging from 29 to 70 years with mean age of 55 years old.

All patients underwent upper gastrointestinal endoscopy, two Biopsies were taken, one of them was immediately fixed in 10% formalin and processed by conventional methods for paraffin wax embedding and H&E stain to verify an adequate number of stomach or esophagus cancer cells were present, other one was collected in normal saline to extract the DNA for detection of EBNA-1 gene of EBV using PCR. SPSS version 16 computer program was used to analyze the data and results.

Out of 39 patients with upper GIT cancer, 22(56.4%) patients were males and 17(43.6%) patients were females. EBV expressed in 2(5.1%) of males and 5(12.8%) of females. 21 (53.8%) were stomach cancer, EBV was positive among 5 of the cases (12.8%), 18 (46.2%) of the demonstrated samples were diagnosed as esophagus cancer , only 2 samples (5.1%) showed positivity for EBV.

On the basis of these findings the study concluded that; the expression of EBV in upper GIT malignancies is statistically insignificant P. value = 0.303.

## ملخص الاطروحة

هذه دراسة وصفية استعافية أجريت في المعمل المركزي -السودان خلال الفترة بين فبراير عام 2012 حتى يوليو 2012. تهدف للكشف عن ارتباط فيروس ابشتاين بار مع سرطان المريء والمعدة لدى المرضى السودانيين باستخدام اختبار تفاعل البلمرة التسلسلي. جمعت العينات من 39 مريضا (21 مشخصين بسرطان المعدة و 18 بسرطان المريء) أعمارهم تراوحت بين 29 إلى 70 عاما بمتوسط عمر 55 سنة.

وقد أجريت لجميع المرضى منظار الجهاز الهضمي، اخذت عينتين خزعة من كل مريض تم وضع واحدة منها فورا في 10% من الفورمالين ومعالجتها بواسطة الأساليب التقليدية لتضمين شمع البارافين وصبغها للتحقق من وجود عدد كاف من خلايا سرطان المعدة أو المريء ، والآخرى جمعت في محلول ملحي لاستخلاص الحمض النووي والكشف عن وجود جين ابشتاين بار مولدات المضادات الفيروسية النووية - 1 باستخدام اختبار تفاعل البلمرة التسلسلي.

(SPSS) تم تحليل البيانات التي تم الحصول عليها باستخدام برنامج التحليل الاحصائي

من أصل 39 مريضا بسرطان الجهاز الهضمي العلوي، 22 (56.4%) من المرضى كانوا من الذكور و 17 (43.6%) من الاناث. كشف عن ظهور فيروس ابشتاين بار في 2 (5.1%) من الذكور و 5 (12.8%) من الاناث، من اصل 21 (53.8%) حاله من سرطان المعدة كشف عن ايجابيه وجود الفيروس في 5 (12.8%) ومن 18 (46.2%) حاله بسرطان المريء وجدت فقط حالتين (5.1%) ايجابيه لهذا الفيروس

على أساس هذه النتائج ، خلصت الدراسة إلى أن الارتباط بين سرطان الجهاز الهضمي العلوي وفيروس ابشتاين بار ليس ذو دلالة احصائية.

<b>Subjects</b>	<b>Page number</b>
الآد	i
<b>Dedication</b>	ii
<b>Acknowledgments</b>	iii
<b>Abstract(English)</b>	v
<b>ملخص الاطر وحة</b>	vi
<b>List of contents</b>	vii
<b>List of Abbreviations</b>	xi
<b>List of tables</b>	xiii
<b>List of Photomicrographs</b>	xiv
<b>CHAPTER ONE</b>	
1.1. Introduction	1
1.2. Objectives	5
<b>CHAPTER TOW</b>	
2. Review of Literature	6
2.1. Anatomy and histology of upper GIT	6
2.1.1. Esophagus	6
2.1.2. The Stomach	7
.2. Pathology of Upper GIT	8
2.2.1. Inflammation and benign condition of the esophagus	8

2.2.1.1. Barrett's Esophagus	8
2.2.1.2. The Esophagitis	8
2.2.1.3. Eosinophilic Esophagitis	9
2.2.1.4. Esophageal Stricture	9
2.2.1.5. Schatzki's Ring	9
2.2.1.6. Esophageal Varicies	9
2.2.1.7. Esophageal Motility Disorders	10
2.2.1.8. Achalasia	10
2.2.1.10. Esophageal webs	10
.2.2. Inflammation and benign condition of stomach	11
2.2.2.1. Peptic ulcer	11
2.2.2.2. Gastroenteritis	12
2.2.2.3. Menetrier disease (hypertrophic gastropathy)	12
2.2.2.4. Hereditary diffuse gastric cancer	12
2.2.2.5. Hereditary non-polyposis colorectal cancer	12
2.2.2.6. Familial adenomatous polyposis (FAP)	13
2.3. Neoplasms	13
2.3.1. Cancer of esophagus	13
2.3.1.1. The signs and symptoms of esophageal cancer	14
2.3.1.2. Types of Esophageal Cancers	14
2.3.1.2.1. Squamous cell carcinoma	14
2.3.1.2.2. Adenocarcinomas	14

2.3.2. Cancer of stomach	14
2.3.2.1. The signs and symptoms of stomach cancer	15
2.3.2.2. Types of stomach cancers	15
2.3.2.2.1. Adenocarcinoma	15
2.3.2.2.2. Lymphoma	16
2.3.2.2.3. Gastrointestinal stromal tumor	16
2.3.2.2.4. Carcinoid tumor	16
2.3.3. Etiology of Esophagus and Stomach cancer	16
2.3.3.1. Age and Sex	16
2.3.3.2. Diet	16
2.3.3.3. Tobacco use	17
2.3.3.4. Alcohol	17
2.3.3.5. Body mass index	17
2.3.3.6. Obesity	17
2.3.3.7. Previous stomach surgery	18
2.3.3.8. Pernicious anemia	18
2.3.3.9. Type A blood	18
2.3.3.10. Inherited cancer syndromes	18
2.3.3.11. BRCA1 and BRCA2	19
2.3.3.12. Inheritance	19
2.3.3.13. Helicobacter pylori infection	19
2.3.3.14. Epstein-Barr virus	19

2.3.3.14.1. EBV and Cancer	21
2.3.3.14.2. Molecular biology of Epstein-Barr virus	23
2.3.3.14.3. Strains of Epstein-Barr virus	23
2.3.3.14.3.1. EBV latent proteins	23
2.3.3.14.3.1.1. EBNA-1	24
2.3.3.14.3.1.2. EBNA-2	24
2.3.3.14.3.1.3. EBNA-3A, EBNA-3B and EBNA-3C	25
2.3.3.14.3.1.4. EBNA-LP	25
2.3.3.14.3.2. Latent Membrane Protein (LMPs)	25
2.3.3.14.3.2.1. LMP1	25
2.3.3.14.3.2.2. LMP2	25
2.3.3.14.3.3. EBERs	26
2.3.3.14.4. The history of EBV infection	26
2.3.3.14.5. Pathogenesis of EBV	27
2.3.3.14.6. Virus and host interactions at molecular level	28
2.3.3.14.7. Immunity of EBV	28
2.3.3.14.8. Oncogenic feature of EBV	29
2.3.4. Diagnoses of EBV associated cancer	30
2.3.4.1. Hematoxylin and eosin (H & E)	30
2.3.4.2. Immunohistochemistry	30
2.3.4.3. Polymerase Chain Reaction Technique (PCR)	30
2.3.4.4. In situ hybridization	30

2.4. Polymerase Chain Reaction (PCR)	31
2.5. Previous study	31
<b>CHAPTER THREE</b>	
3. Materials and Methods	36
3.1. Study Design	36
3.2. Study area	36
3.3. Study population and Sample size	36
3.4. Data collection	36
3.5. Tissue sample collection	36
3.6. Histopathology	36
3.6.1 Upper Gasterotestinal Endoscopy and Biopsy specimens	36
3.6.2 Sample preparation	37
3.6.3. Hematoxylin and Eosin stain	37
3.7. Polymerase chain reaction (PCR)	37
3.7.1. DNA extraction	37
3.7.1.1. Principle	37
3.7.1.2. Procedure	38
3.7.2. Oligonucleotides (Primers)	38
3.7.3. PCR amplification	39
3.7.4. Analysis of PCR product	39
3.8. Statistical analysis	40
3.9 Ethical consideration	40

<b>CHAPTER FOUR</b>	
4. Result	41
<b>CHAPTER FIVE</b>	
5. Discussion	52
<b>Conclusion and Recommendations</b>	
Conclusion	54
Recommendations	54
<b>References</b>	
<b>Appendix</b>	

## List of Abbreviation and Symbols

%	percent
C	Degree Centigrade
CD	Cluster of Differentiation
dATP	Deoxyadenosine Triphosphate
dCTP	Deoxycytidine Triphosphate
ddH <sub>2</sub> O	Double distilled water
dGTP	Deoxyguanosine Triphosphate
DNA	Deoxyribonucleotide
dNTP	Deoxynucleotide Triphosphate
D.P.X	Polystyrene resin dissolved in xylene
dTTP	Deoxythymidine Triphosphate
EBERs	Epstein-Barr virus-encoded RNAs
EBNA	Epstein-Barr virus Nuclear Antigen
EBNA-LP	Epstein-Barr virus Nuclear Antigen-Leader protein
Et-Br	Ethidium bromide
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
H&E	Haematoxylin and Eosin
HPV	Human Papilloma Virus
HTLV	Human T Lymphocyte Virus

ICAM-1	Intercellular Adhesion Molecule-1
IHC	Immunohistochemistry
IL	Interleukin
Kb	Kilo-base
Kbp	Kilo-base pair
KDa	KiloDalton
LFA-1	Leukocyte Function-Associated Antigen-1
LMP1	Late Membrane Protein
LP	Lymphocyte Predominant
M	Molar
Mg	Milligram
MCH	Major Histocompatibility Complex
ML	Milliliter
mM	Millimole
MØ	Macrophage
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
RPM	Round per Minute
TBE	Tris-Base Boric EDTA
UV	Ultra Violet
µL	Microliter

## LIST OF TABLE

NO. Table	Title	Page
1	<b>Shows distribution of the study population by age.</b>	43
2	<b>Shows relationship between type of cancer and age of the study populated.</b>	44
3	<b>Shows the distribution of cancer types according to gender.</b>	45
4	<b>Shows the expression of EBV among different age groups of the studied subjects.</b>	46
5	<b>The expression of EBV among gender of the studied subjects.</b>	47
6	<b>Shows the association between type of cancer and EBV positivity.</b>	48

## LIST OF PHOTOMICROGRAPHS

NO. Photomicrographs	Title	Page
1	<b>Display adenocarcinoma of stomach in slide stained by H&amp;E (x40).</b>	<b>49</b>
2	<b>Display poorly differentiated squamous cell carcinoma of esophagus in slide stained by H&amp;E (x40).</b>	<b>50</b>
3	<b>Amplification of Epstein-Barr virus by Epstein-Barr nuclear antigen-1 (EBNA-1) gene with 80 bp.</b>	<b>51</b>