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Molecular and Immunological Study of *Human T-Lymphotropic Virus Type I*: (*HTLV-I*) in Iraqi Patients with Brain Tumors

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

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Certification

*I certify that this thesis entitled (**Molecular and Immunological Study of Human T- Lymphotropic Virus Type I: (HTLV-I) in Iraqi Patients with Brain Tumors)** was prepared By **Ataa Khalil Hussein Jasem Al-Shamari** under my supervision at the Department of Biology, College of Science / University of Babylon, as a partial fulfillment of the requirements for the degree of Doctorate of Philosophy in Biology.*

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2023

Dedication

To:

The best who supported and encouraged me ...

My Father

The source of inspiration and gave me strength, who continually provides moral, spiritual, and emotional support....

My Mother

To my beloved and my fortune in life, my sister and brother...

Jumana & Rasool

To my beloved family, I dedicate this work, which forms the future of my life...

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2023

Summary

Summary:

Human T-cell lymphotropic virus type-I (*HTLV-1)* is the etiologic agent of adult T-cell leukemia/lymphoma and *HTLV-1*-associated myelopathy/tropical spastic paraparesis. Cytokines are proposed to play important roles in brain tumor biology, neurodegeneration, or impaired neuronal function. Gene polymorphism in the TNF-α and IL-1 may affect susceptibility to brain disease. IFN-γ is best known for its ability to regulate host immune responses. KIR and IL-28 have the ability to induce resistance to viral infection.

This study was aimed to determine the percentage of HTLV-1 and whether polymorphisms at TNF-α, IFN-γ, KIR, IL-1Ra, and IL-28 locus modulate the risk of developing brain tumors in a group of Iraqi population samples. A cross-sectional case-control study included one hundred and fifty (150) specimens enrolled in the current research, including seventy-five (75) randomly selected blood and brain tumor tissues with different types and grades related to those aged 2 years to 85 years. Additionally, twenty-five (25) autopsies were collected from non-brain tumor control cases aged 16 to 71 years, which were obtained from deceased individuals with non-neurological causes, showing normal brain histology as a baseline control group. Fifty blood specimens from apparently healthy individuals were recruited as a control group from different Teaching Hospitals in the Mid-Euphrates and Baghdad Governorates of Iraq. The specimens were collected during the period from January to September 2022.

Brain tissues, as well as three to five ml of blood sample, were collected, and then RNA extraction; total DNA extraction as well as separation of blood serum were done and stored at -70°C till used. The first part of the work was done for HTLV-1 detection by Real-time- PCR technique. While, the second part was done

for the estimation of TNF-α, IFN-γ, KIRs, IL1Ra, and IL-28 genes polymorphisms by Sanger sequencing. Moreover, used ELISA technique to evaluate the serum levels of TNF-α, IFN-γ, KIRs, IL1Ra, and IL-28 in patients with brain tumors and apparently healthy control group.

The tissue samples were related to patients with brain tumors with a mean age of 50.7 + 10.4 years, as compared to 47.9 + 12.6 years for their control counterparts. Males accounted for 41 (54.7%) of brain tumor cases, while females accounted for 34 (45.3 %). The ratio of males to females was 1.2:1, while the control group had a sex distribution of 45 (60%) males and 30 (40%) females.

Grade I was found in 37 cases (49.3%) of brain tumor cases (20 males and 17 females), while grade II was found in 17 cases (22.7%) (9 males and 8 females), grade IV was seen in 13 cases which were (17.3%) (7 males and 6 females); and finally, grade III were seen in 8 cases only (10.7%) (5 males and 3 females).

Twenty-one (21) cases of brain tumors were as Pilocytic Astrocytoma (28 %), 15 cases as Meningotheliomatous Meningioma (20%), 13 cases as Glioblastoma Multiforme and Diffuse Fibrillary Astrocytoma (17.3%) for each one, 6 cases as Anaplastic Oligodendroglioma (8%), 4 cases as Atypical Meningioma (5.4 %), and 3 cases as Transitional Meningioma (4%), these data showed a substantial statistical difference (P= 0.04) between brain tumors.

In this study, a viral infection of brain tumors was positive in 50 (66.7%) and negative in 25 (33.3%) out of 75 patients. While only 5 (6.6%) of the control group (3 from blood out of 50 cases as well as 2 from brain tissues out of 25 cases) have viral genome. Statistically significant differences (p = 0.01) among patients group. The positive result of HTLV-1 - qRT-PCR shows 42 % (21 out of 50 cases) as positive while 58% (29 out of 50 cases) as negative, whereas no positive signals for HTLV-1 in the control group.

The most infected brain tumor tissues with RNA-*HTLV-1* are related to the age stratum (70-85 years), which accounted for 33.3 % (7 out of 21 tissues). The percentage of brain tumor tissues that have positive *HTLV-1* -RT-PCR results based on the sex of patients, showed that males accounted for 61.9% (13 out of 21 cases) and females accounted for 38.1% (8 out of 21 cases).

Positive HTLV-1 qRT-PCR detection results in patients with various forms of brain tumor types were 10%, 6%, 18%, 4%, and 4% for Pilocytic Astrocytoma; Meningotheliomatous Meningioma; Glioblastoma Multiforme; Diffuse Fibrillary Astrocytoma and Transitional Meningioma, respectively.

TNF-α gene polymorphism distribution of GG; GA and AA were 40%, 30%, and 30%, respectively, in patients with brain tumors and 80%; 4% and 16%, respectively, in the control group. Also, this study has documented a new recordings in NCBI and Gene Bank under accession number: BankIt2620154: OP380425; OP380426; OP380427; OP380428; OP380429; OP380430; OP380431; OP380432.

IFN-γ gene polymorphism percentage of CC; CA and AA were 44%, 34%, and 22%, respectively, in patients with brain tumors and 72%; 12% and 16%, respectively in the control group. Also, this study has documented a new recordings in NCBI and Gene Bank under accession number: Bank It 2617649: OP326723; OP326724; OP326725; OP326726; OP326727; OP326728; OP326729; OP326730; OP326731.

KIRs gene polymorphism frequency of TT; AT and AA were 38%, 32%, and 30%, respectively, in patients with brain tumors and 76%; 8% and 16%, respectively in the control group. Also, new recordings were documented in NCBI and Gene Bank under accession number: BankIt2619196 OP373670; OP373671; OP373672; OP373673; OP373674; OP373675; OP373676; OP373677.

IL1Ra rs2234679 gene polymorphism distribution according to CC; CG and GG were 40%; 30% and 30%, respectively, in patients with brain tumors and 68%, 12%, and 20%, respectively in the control group. While the results of IL1Ra **rs16065** polymorphism according to TT; TC and CC were 54%, 16%, and 30%, respectively, in patients with brain tumors and 60%; 12% and 28%, respectively in the control group. In addition, new recordings were documented in NCBI and Gene Bank under accession numbers: BankIt2618097; OP351527; OP351528; OP351529; OP351530; OP351531; OP351532; OP351533; OP351534; OP351535; OP351536.

IL-28A gene polymorphism frequency according to GG; GC and CC were 46%; 20% and 34%, respectively in patients with brain tumors and 64%; 20% and 16%, respectively in the control group. Also, new recordings in NCBI and Gene Bank were documented under accession number: BankIt2618865: OP359987; OP359988; OP359989; OP359990; OP359991; OP359992; OP359993; OP359994; OP359995; OP359996.

Statistically, a significant difference ($p<0.05$) was found on comparing the mean of serum TNF-α IFN-γ, KIR, and IL-1Ra among study groups. While non-significant difference ($p<0.05$) was found in comparing the mean of serum level of IL-28 among these study groups.

The conclusions of the current study are: *HTLV-1* could be acted as a cofactor in pathogenesis as well as the development biology of brain tumors in Iraqi patients. Gene polymorphism associated with high serum levels of TNF-α, IFN-γ, and KIRs may play roles as a risky factor in the pathogenesis of idiopathic Brain Tumors. In contrast, IL-1Ra may play as a protective factor in Iraqi patients with brain tumors. Lastly, non-medical associations for IL-28 in patients with brain tumors cannot be useful as diagnostic factors in patients with brain tumors.

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List of Abbreviations

Abbreviated Form	Meaning
AHC	Apparently Healthy Control
AIDS	Acquired Immunodeficiency Syndrome
AP-1	Activator Protein 1
APCs	Antigen Presenting Cells
ARMS-PCR	Amplification Refractory Mutation System-Polymerase Chain Reaction.
ATL	Adult T-Cell Leukemia/Lymphoma
AYA	Adolescents And Young Adults
BD	Bipolar Disorder
Bax	Bcl-2-Associated X Protein
BBB	Blood–Brain Barrier
Bcl-x	B-Cell Lymphoma-Extra Large
BLASTn	Basic Local Alignment Search Tool Nucleotide
CBP/p300	Camp Response Element Binding Protein (CREB) Binding Protein
CBTRUS	The Central Brain Tumor Registry Of The United States
CCL22	C-C Motif Chemokine Ligand 22
CCR4	CD4+ Lymphocyte Receptor
CMIA	Chemiluminescent Microparticle Immunoassay
CMV	Cytomegalovirus
CNS	Central Nervous System
CREB/ATF	Cyclic Amp Response Element-Binding Protein/Activating Transcription Factor
CSF	Cerebrospinal Fluid
CTLs	Cytotoxic T Lymphocytes
CXR	Carboxy-X-Rhodamine
DAAs	Direct Acting Antivirals
DALY	Disability-Adjusted Life Years
dbSNP	The Single Nucleotide Polymorphism Database
dNTPs	Deoxynucleotide Triphosphates
EBV	Epstein-Barr Virus
EGFR	Epidermal Growth Factor Receptor
ELISA	Enzyme-Linked Immunosorbent Assay
Env	Envelope Protein
EtOH	Ethyl Alcohol
Gag	Group-Specific Antigen
GBM	Glioblastoma Multiforme
G-CSF	Granulocyte-Colony Stimulating Factor
GLUT-1	Glucose Transporter 1
HAM/TSP	HTLV-1 -Associated Myelopathy/Tropical Spastic Paraparesis
HBZ	HTLV-1 basic leucine zipper factor

HCC	Hepatocellular Carcinoma
HHV-6	Human Herpesvirus-6
HIV	Human Immunodeficiency Virus
HIVE	HIV Encephalitis
HLA	Human Leukocyte Antigen
HRP	Horseradish Peroxidase
HSPG	Heparan Sulfate Proteoglycan
hTERT	Hot Genes Telomerase Reverse Transcriptase
HTLV-1	Human T-Cell Lymphotropic Virus Type 1
ICAM-1	Intercellular Adhesion Molecule 1
IDH	Isocitrate Dehydrogenase
IDO	Indoleamine-2,3-Dioxygenase
IDUs	Injecting-Drug Users
IFN- γ	Interferon-Gamma
IFN-λ	Interferon Lambda
iKIRs	Inhibitory Killer Cell Immunoglobulin-Like Receptors
IL	Interleukin
IL-1R	Interleukin (IL)-1 Receptor
IL-1Ra	Il-1 Receptor Antagonist
IL-1RN	Interleukin-1 Receptor Antagonist Gene
IL-1α	Interleukin-1 Alpha
IL-1β	Interleukin-1 Beta
IL-28	Interleukin 28
IN	Integrase
JAK/STAT	Janus Kinase/Signal Transducers And Activators Of Transcription
JC virus	John Cunningham Virus
KC	Kupffer Cell
kDa	Kilodalton
KIR	Killer Immunoglobulin-Like Receptors
KIR2DL1	Killer Cell Immunoglobulin Like Receptor, Two Ig Domains And Long Cytoplasmic Tail 1
KIR2DP1	Killer Cell Immunoglobulin Like Receptor, Two Ig Domains Pseudogene 1
KIR2DS4	Killer Cell Immunoglobulin Like Receptor, Two Ig Domains And Short Cytoplasmic Tail 4
LRC	Leukocyte Receptor Complex
LTR	Long Terminal Repeats
MA	Matrix
MAPK	Mitogen-Activated Protein Kinases
MHC	Major Histocompatibility Complex
MHV	Mouse Hepatitis Virus
NC	Nucleocapsid
NCBI	National Center For Biotechnology Information
NES	Nuclear Export Sequence
NFAT	Nuclear Factor Of Activated T-Cells

NFκB	Nuclear Factor-Kappa B
NK cells	Natural Killer Cells
NKT cells	Natural Killer T Cells
NLS	Nuclear Localizing Sequence
NRP-1	Neuropilin 1
OD	Optical Density
OR	Odds Ratio
ORF	Open Reading Frame
PCNA	Proliferating Cell Nuclear Antigen
Pd-L1	Programmed Cell Death Ligand 1
PM	Particulate Matter
Pol	Polymerase
Pro	Protease
PX	Protein X
qPCR	Quantitative PCR
Rex	Regulator X
RF-EMR	Radiofrequency Electromagnetic Radiation
RT	Reverse Transcriptase
rt-PCR	Real Time Polymerase Chain Reaction
RxRE	Rex Response Element
SAH	Subarachnoid Hemorrhage
SDF-1	Stromal Cell-Derived Factor-1
sHBz	Spliced HTLV-1 Basic Leucine Zipper Factor
SLE	Systemic Lupus Erythematosus
SNPs	Single Nucleotide Polymorphisms
SSPs	Sequence-Specific Primers
SVR	Sustained Virological Response
Tax	Transactivator Protein
TBE	Tris/Borate/Edta
TGF-β	*Transforming Growth Factor-Beta*
TM	Transmembrane
TME	Theiler's Murine Encephalomyelitis Virus V
TNF-α	Tumor Necrosis Factor
TRAF6	TNF Receptor Associated Factor 6
TREs	Tax Responsive Elements
VNTR	Variable Number Of Tandem Repeats
WHO	World Health Organization
Wnt	Wingless-Related Integration Site

Chapter One

Introduction

1. Introduction

Brain tumors develop a complex tumor microenvironment, contributing to drug resistance development. In addition to tumor cells, non-neoplastic cells such as astrocytes, microglial cells, endothelial cells, and lymphocytes are present in brain tumor microenvironment. Communications between cancer and non-neoplastic cells play critical roles in tumorigenesis and tumor invasion. Among the non-neoplastic cells, and microglial cells account for 30–50% of the total brain tumor mass (Wu and Watabe, 2017; Abolanle *et al*., 2020).

Many viral infections cause acute and chronic neurologic diseases, leading to cortical function degeneration. While neurotropic viruses that gain access to the central nervous system (CNS) may induce brain injury directly via infection of neurons or their supporting cells, they also alter brain function via indirect neuroimmune mechanisms that may disrupt the blood-brain barrier (BBB), eliminate synapses, and generate neurotoxic astrocytes and microglia that prevent recovery of neuronal circuits. Non-neuroinvasive, neurovirulent viruses may also trigger aberrant responses in glial cells, including those that interfere with motor and sensory behaviors, encoding of memories, and executive function. Neuroprotective antiviral responses that amplify levels of innate immune molecules dysregulate normal neuroimmune processes, even in the absence of neuroinvasion, which may persist after the virus is, cleared (Davé and Klein, 2023).

Human T-cell lymphotropic virus type 1 (*HTLV-1*) is an exogenous retrovirus endemic to some tropical regions. *HTLV-1* reverse transcribes its viral RNA genome into double-stranded DNA, which is then integrated into the host genomic DNA, forming a provirus, which serves as a template for generating new viral particles. A characteristic of *HTLV-1* infection is that the virus maintains its copy

number during chronic infection not via the production of free viral particles but via clonal expansion and persistence of infected T-cell clones (Matsuo *et al*., 2022).

HTLV-1 is a highly oncogenic virus that manipulates host cellular signaling pathways to induce cancer hallmarks with successful immune surveillance evasion. Viral gene products and their interaction with host proteins to alter their function and thus favor viral infection and persistence mediate the oncogenic ability of *HTLV-1*. Several mechanisms involving Tax and HBZ have been demonstrated to play a role in *HTLV-1*-induced oncogenesis (Mohanty and Harhaj, 2020; Einsiedel *et al*., 2021).

HTLV-1 is the etiologic agent of acute, chronic, and inflammatory disorders. Among these, an aggressive $CD4^+$ T-cell malignancy, adult T-cell leukemia/lymphoma (ATL), and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) are significant life-threatening morbidities (Hedayati *et al*., 2022).

With a focus on their potential significance in survival, numerous studies have investigated the impact of single nucleotide polymorphisms (SNPs) in genes associated with inflammation on the risk of developing glioma. Certain SNPs in cytokine genes affect the expression of corresponding cytokines, which may interfere with individuals' susceptibility to different types of malignancies (Sharman *et al*., 2015; Ding *et al*., 2020).

Numerous data on host genetic variations associated with immune responses to *HTLV-1* infection, including human leukocyte antigen (HLA), killer immunoglobulin-like receptors (KIR), IL-6, IL-10, IL-28, Fas, Fas ligand, IFN- γ, TNF-α, and mannose-binding lectin, have been described as potential biomarkers

associated with progression from infection to disease (Assone *et al*., 2018; Vallinoto *et al*., 2019).

TNF-α is a pro-inflammatory cytokine secreted by monocytes, macrophages, activated natural killer (NK) cells, and T-cells. In a healthy brain, TNF-α is responsible for dendritic cell (DC) maturation, whereas, in tumorigenic conditions, its expression correlates with Brain tumor grade. In the glioma microenvironment, TNF-α secretion leads to the promotion of tumor formation and angiogenesis. Thus, TNF-α enables glioma cells to escape from immune response and grow aggressively in the inflammatory microenvironment. TNF-α also plays a significant role in tumor growth by activating macrophages through SDF-1 induction to attack T-cells and other immunogenic factors (Mostofa *et al*., 2017; Sheng *et al*., 2018).

In tumors, IFN-γ is an influential apoptosis-inducing factor by directly inducing caspase-1 and caspase-8 in tumor cells. IFN-γ also plays a vital role in regulating immune response; antigen presentation; inflammation; chemotactic signaling; and modulating extracellular matrix, thereby affecting metastasis and tumor structure; and activation and polarization of white blood cells (Glasner *et al*., 2018). Furthermore, IFN-γ plays an essential role in inducing PD-L1 expression in glioma (Burke and Young, 2019).

Killer-cell immunoglobulin-like receptors (KIR) play an essential role in the signaling of natural killer cells, which are involved in the activity of CD8+ T cells; therefore, these have significant repercussions in the innate and adaptive immune response. KIR tends to suffer genetic mutations, indicating a high polymorphic capacity. They are expressed on the surface of NK cells and certain T lymphocytes and regulate the function and development of these cells (Wawina-Bokalanga *et al*., 2021).

IL-1 receptor antagonist (Anakinra) has been investigated in exploratory studies in selected human tumors, including colorectal and breast carcinoma. Blockage of IL-1 signaling had a neuroprotective effect and improved the neurologic recovery after traumatic brain injury. Moreover, administration of IL-1Ra proved to reduce the inflammatory response in animal models of brain ischemia (Yates *et al*., 2021).

IL-28 (coding for IFN-λ) is an important cytokine that is responsible for an unspecific antiviral response by interacting with the HLA class II receptor, inducing intracellular signaling by Janus kinase/signal transducers and activators of transcription (JAK/STAT) and mitogen-activated protein kinases (MAPK). Host genetic background in HLA class II, encoded by single nucleotide polymorphisms (SNPs), can lead to a spatial conformation in the receptor, modifying the attachment that avoids interaction between IFN-λ and its receptor, inducing a genetic by-stand interaction. The secretion of IL-28 within the tumor microenvironment promoted NK cell activation in vivo and sensitized tumors to NK cell recognition and killing (Zhang *et al*., 2021).

1.1. Aim of Study

Considering all these points, this research study was designed for the detection of the rate of *Human T- Lymphotropic Virus type I* (*HTLV-I*) infection among a group of Iraqi patients with brain tumors and autopsies from the adults non-brain tumors and their correlation with TNF-α, IFN-γ, KIR, IL1Ra, and IL-28 genes polymorphism, **through achieving the following objectives:**

1. To determine the percentage of *HTLV-I* in brain tumor specimens that range from autopsies of non-brain tumors adults to patients suffering from brain tumors by molecular techniques such as rt-PCR.
2. To estimate the genetic polymorphism of TNF-α, IFN-γ, KIR, IL1Ra, and IL-28 genes in patients with brain tumors and control groups.

3. To statistically compare the levels of serum of TNF-α, IFN-γ, KIR, IL1Ra, and IL-28 in patients with brain tumors and apparently healthy individuals by ELISA.
4. To find out possible correlation and the association between these TNF-α, IFN-γ, KIR, IL1Ra, IL-28, and *HTLV-I* in patients with brain tumors and control groups.

Chapter Two

Literatures Review

2. Literatures review

2.1. Brain Tumor

Brain tumor is one of the most malignant tumors causing death, represents the 17th most common cancer worldwide, constitutes 2-3% of all malignant neoplasms and nearly 85-90% of all primary CNS tumors, with an estimated five-year survival rate of up to 35% for malignant tumors and about 90% for benign tumors (Neugut *et al*., 2019; Sajjad *et al*., 2019). It accounts for approximately 1.35% of all malignant neoplasms and 29.5% of cancer-related death (Lapointe *et al*., 2018).

Brain and CNS tumors include tumors of the brain, cranial nerves, spinal nerves, spinal cord, and the meninges. The tumor can be broadly classified as malignant and non-malignant (or benign) (Clifton and Reimer, 2019). The most common malignant brain tumor is glioblastoma, originating from glial cells (Udaka and Packer, 2018).

2.1.1. Types of brain tumors

1- Primary brain tumor

Primary brain tumors - A tumor that begins in the brain is called a primary brain tumor. It can be either (Van Maele-Fabry *et al*., 2019):

a- Benign: This type of tumor has slow growth and is defined by distinct boundaries.

b- Malignant: This type of tumor has rapid growth and irregular boundaries. It spreads nearby.

2- Secondary brain tumor

A cancerous tumor, which starts from another part of the body and ends up in the brain, is a secondary or metastatic brain tumor; carrying cancerous cells by the bloodstream to the brain that develops this type of effect. The most commonly

observed cancer type is the lung and breast cancer. For example, the lung cancer cell can spread to the brain and cause tumors (Peddinti *et al*., 2021).

2.1.2. Grades of brain cancer

World Health Organization (WHO) grades brain tumors from I-IV based on their catastrophic potential. The title is low-grade tumors I and II, whereas grades III and IV are malignant or high-grade, leading to serious complications (Kim, and Lee, 2022).

Table (2.1): WHO classification of brain tumor grades (Kim, and Lee, 2022).

	Grade	Tumor Types	Characteristics
Low Grade	Grade I	• Craniopharyngioma • Chordomas • Ganglioglioma • Gangliocytoma • Pilocytic astrocytoma	• Possibly curable via surgery alone • Long-term survival • Least malignant (benign) • Non-infiltrative
	Grade II	• Pineocytoma • “Diffuse” astrocytoma • Pureoligodendroglioma	• Slight infiltrative • Relatively slow growing • Can recur as higher grade
High Grade	Grade III	• Anaplastic ependymoma • Anaplastic astrocytoma • Anaplastic oligodendroglioma	• Malignant • Infiltrative • Tend to recur as a higher grade
	Grade IV	• Glioblastoma multiforme • Medulloblastoma • Ependymoblastoma • Pineoblastoma	• Most malignant • Rapidly growing and aggressive • Widely infiltrative • Recurrence • Tendency for necrosis

2.1.3. Risk Factors

I. Ionizing radiation (X-rays and gamma rays):

Ionizing radiations can potentially induce brain tumors, especially meningiomas, gliomas, and nerve sheath tumors. The carcinogenic effects of ionizing radiation are more potent in children, as they are more radiosensitive and have more years of potential life to express the risk. The risk was higher for younger children, and the latency between irradiation and brain tumor occurrence has been estimated at 7-9 years, with meningiomas and gliomas being the predominant induced tumor types (Ostrom *et al*., 2019).

II. Radiofrequency electromagnetic radiation (RF-EMR)

Mobile phones emit low-energy RF-EMR; when used against the head, the brain absorbs the most significant dose. Due to public health concerns, the association between the risk of brain tumors and cellular phone use has been investigated extensively (Pareja-Peña *et al*., 2022).

Free radicals in the brain have strong cytotoxic properties; RF-EMR exposure generates free radicals inside the cell, which damages the DNA strands and destroys normal cell functioning. The glial cells are more likely to switch into cancerous cells by DNA damage (Kim *et al*., 2019).

III. Obesity

Chronic low-grade inflammation within the brain increasingly appears to be a key component in severe obesity, strongly connected with the metabolic dysregulations that classically characterize this condition (Capuron *et al*., 2017).

IV. Genetic factors

Several oncogenes (growth factors) are involved in the progression of brain tumors. Tumor progression is strongly linked with certain mutant inherited genes, including p53 genes, mutant epidermal growth factor receptor (EGFR) gene is associated with high-grade tumors, and mutation in IDH2 and IDH1 genes are associated with tumor aggression and progression (Vijapura *et al*., 2017; Alavi and Hamidi, 2019).

V. Pesticides

Some pesticides, including carbamate and insecticides, have been proven to be potent brain carcinogens (Vidart *et al*., 2019; Piel *et al*., 2019)

VI. Air pollution

Outdoor air pollution comprises several potentially carcinogenic factors: particulate matter (PM), black carbon, heavy metals (e.g., vanadium, nickel, and manganese), environmental tobacco smoke, organic compounds (e.g., polycyclic aromatic hydrocarbons and endotoxins) and gaseous pollutants (e.g., O3, NO2, SO2, CO). PM and O3 are thought to be the 2 most important dangers to public health (Vienne-Jumeau *et al*., 2019). Brain inflammation within a tumor microenvironment is thought to increase oxidative stress and DNA damage, thus stimulating both genetic and epigenetic changes during glioma evolution (Maher, 2016).

VII. N-nitroso compounds (NOCs)

In humans, continuous use of dietary NOCs has carcinogenic activity (Turesky, 2018).

VIII. Epigenetics

Epigenetics is defined as the alterations in the gene expression profile of a cell that are not caused by changes in the DNA sequence, which refers to the study of heritable modifications in chromosomes. In glioma brain tumors, global genome hypo methylation contributes to the tumor progression through three mechanisms, including loss of imprinting, promoting genomic instability, and activation of oncogenes (Morgan *et al*., 2018).

IX. Viral infection

A viral etiology of specific brain tumors has gained interest, although the role of viruses in oncogenesis remains controversial. Certain viruses, such as adenoviruses, retroviruses, and papovaviruses, can potentially induce brain tumors (Stark and Campbell, 2014).

2.2. *Human T-lymphotropic Virus-1* (*HTLV-1*)

2.2.1. The history of *HTLV-1*

Retroviruses are a unique family of viruses associated with numerous disease manifestations and cancer development in various species. The earliest documentation of diseases caused by retroviruses was in the early 1900 and included the animal diseases equine infectious anemia (1904) and avian leukosis (1908) (Haines *et al*., 2012; Miller *et al*., 2021). In 1911, Rous discovered that he could induce solid tumors in healthy chickens by injecting cell-free extract from tumors from sick chickens. The virus associated with these tumors became the Rous sarcoma virus. Rous sarcoma and avian leucosis viruses are the prototypic infectious agents related to viral-induced neoplasia. Years later, Dr. Temin's work with the Rous sarcoma virus led to the proviral hypothesis, which stated that

although some viruses have an RNA genome, they exist within the host cell as a DNA provirus (Rubin, 2011). His discovery of a unique enzyme, reverse transcriptase, later confirmed this controversial theory, which allowed retroviruses to transcribe their genome "backward" from RNA to DNA. The importance of this finding was acknowledged as Temin and David Baltimore received the Nobel Prize for their discovery of reverse transcriptase in 1975 (Coffin and Fan, 2016).

Two years later, Uchiyama *et al*. (1977) described the first retroviral disease in humans. They discovered an aggressive T-cell malignancy in humans called adult T-cell leukemia (ATL). Subsequently, Poiesz *et al*. (1980) reported virus particles isolated from a cell line derived from patients with a cutaneous T-cell lymphoma. In both the United States and Japan, this new virus was causally linked to ATL, representing not only the first verified human retrovirus but also the first human retrovirus to be directly linked with the development of cancer (Coffin, 2015; Vallinoto *et al*., 2022).

The viruses in the United States and Japan were found to be identical, and the new virus was eventually named *Human T-lymphotropic virus type 1* (*HTLV-1*). Later *HTLV-1* was found to be associated with several other disorders, including a neurologic disease resulting in progressive myelopathy described in French Martinique as Tropical Spastic Paraparesis (TSP) and in Japan as *HTLV-1*-associated myelopathy (HAM) (Bangham *et al*., 2015; Olindo *et al*., 2018).

2.2.2 Taxonomy and Classification

Human T-cell lymphotropic virus type 1 or *human T-cell leukemia virus type 1* (*HTLV-I*), is from the Artververicota phylum; Revtraviricetes class; Ortervirales order; Retroviridae family; Orthoretrovirinae subfamily; Delta retrovirus genus

(Zargari *et al*., 2020), Species Simian T-lymphotropic virus and the Serotypes HTLV-1 (Table 2.2). In 1979, Poiesz and Ruscetti discovered the first human retrovirus, HTLV. The virus was isolated from the patient with a T-cell malignancy. HTLV has infected humans for many decades, but the knowledge of this virus has been later revealed (Vallinoto *et al*., 2022). A second human retrovirus (*HTLV-2*) was discovered after HTLV-1. HTLV-2 was found mostly in North, Central, and South America, as well as Central and West Africa. HTLV-2 has approximately 70% similar genome structure to HTLV-1. HTLV-3 and HTLV-4 were discovered after HTLV-2, but HTLV-1 was interested by scientists (Vicento and Kamangu, 2021; Raza *et al*., 2021).

Human T-cell lymphotropic virus type 1 uses an enzyme called reverse transcriptase to produce DNA from RNA. The DNA is subsequently incorporated into the host's genome. HTLV predominantly affects T lymphocytes (Bangham, 2018).

The envelope, RNA, and proteins are the main components of virion. The proteins comprise protease, gag, env, and pol proteins (Krupovic and Koonin, 2017). Gag proteins are major components of the viral capsid, about 2000-4000 copies per virion. Protease is expressed differently in different viruses. It functions in proteolytic cleavages during virion maturation to make mature gag and pol proteins. Pol proteins are responsible for the synthesis of viral DNA and integration into host DNA after infection. Finally, env proteins play a role in association and virion entry into the host cell (Coffin, 2015).

Human T-cell lymphotropic virus type 1 is associated with several kinds of diseases, including very aggressive adult T-cell lymphoma (ATL), HTLV-I-

associated myelopathy, Uveitis, Strongyloides stercoralis hyper-infection, and some other diseases (Schierhout *et al*., 2020; Ramezani *et al*., 2022).

Table (2.2): Classification of Retrovirus Family (Cameron, and Arnold, 2021)

Subfamily	Orthoretrovirinae
Genera	Alpharetrovirus (e.g., Rous sarcoma virus (chickens))
	Betaretrovirus (e.g., Mouse Mammary Tumor virus)
	Gammaretrovirus (e.g., Leukemia and sarcoma viruses)
	Deltaretrovirus (e.g., Human T-lymphotropic viruses)
	Epsilonretrovirus (e.g., Skin sarcoma virus)
	Lentivirus (e.g., Human Immunodeficiency Viruses, Visna virus (sheep))
Subfamily	Spumaretrovirinae
Genera	Bovispumaviruses (e.g., bovine foamy virus)
	Equispumaviruses (e.g., equine foamy virus)
	Felispumaviruses (e.g., feline foamy virus)
	Prosimiispumaviruses (e.g., brown greater galago prosiminian foamy virus)
	Simiispumaviruses (e.g., eastern chimpanzee simian foamy virus).

2.2.3. Genome organization and replication of HTLV-1

HTLV-1 is a complex retrovirus from the family Retroviridae. The virus particles are 110-140 nm in size and contain the approximately 9 kb positive stranded diploid RNA genome with host cell tRNA needed for the initiation of transcription (Hirons *et al*., 2021). The genome is complexed with viral encoded matrix (MA), capsid (CA), and nucleocapsid (NC) proteins to form the core of the viral particle. It is surrounded by an envelope consisting of two viral proteins (surface unit, SU, and transmembrane unit, TM) embedded into the host cell-derived membrane as in figure (2.1) (Haines, 2012; Eusebio-Ponce *et al*., 2019).

The HTLV-1 genome is approximately 9032 nucleotides in length and contains genes that encode the common retroviral structural proteins, group-specific antigen (Gag), protease (Pro), polymerase (Pol), and envelope (Env) (Martinez *et al*., 2019). As a complex retrovirus, HTLV-1 also contains a 3' pX region that encodes for numerous regulatory and accessory proteins. The pX region was initially considered non-essential in the viral life cycle (Bangham and Matsuoka, 2017).

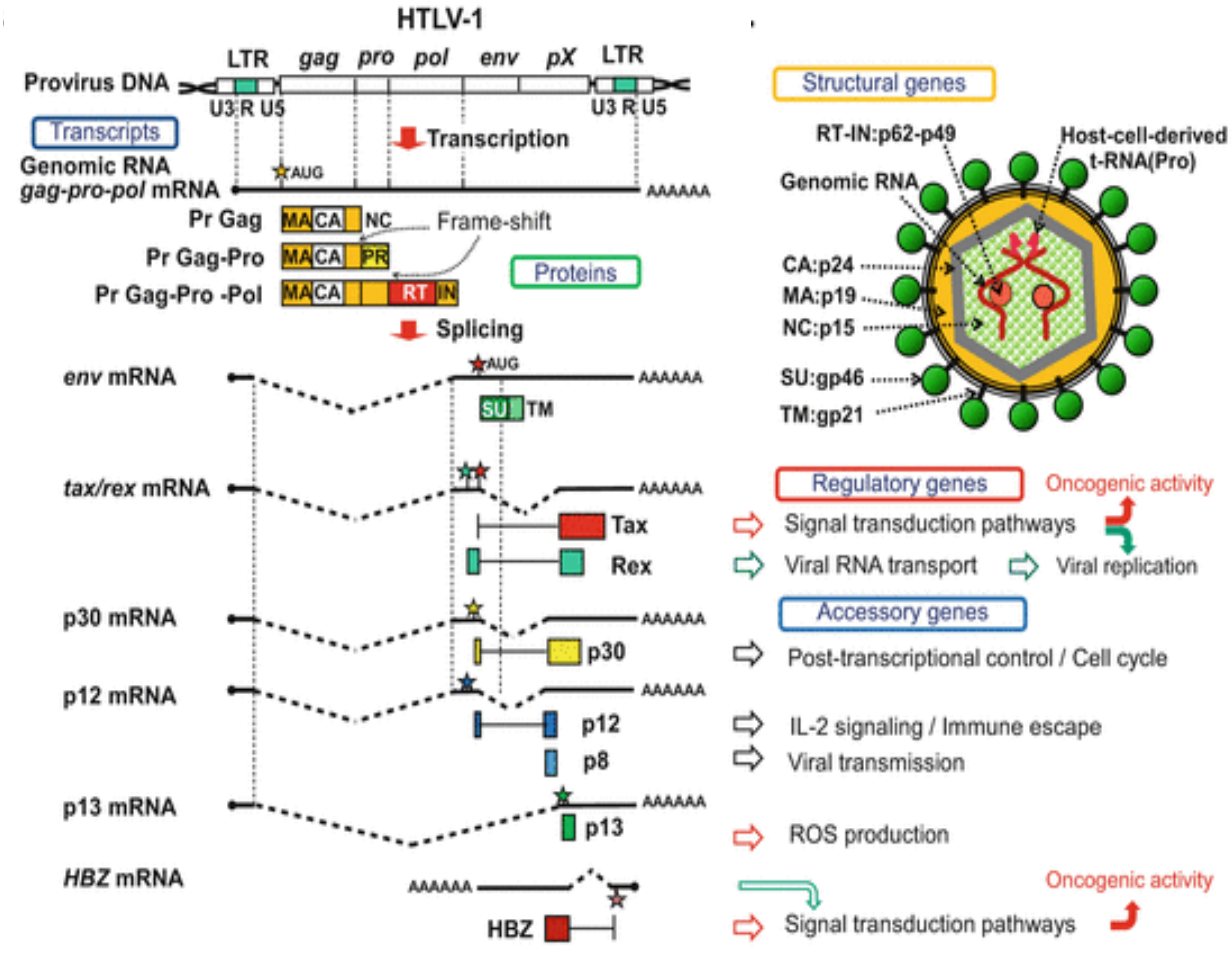

Figure (2.1). Organization of the HTLV-1 genome (Fujisawa, 2017).

The proteins encoded within this region are generated through alternate splicing of viral mRNA and using alternative translation start codons (Volkening *et al*., 2023). The two regulatory proteins, Tax and Rex, are coded in ORF IV and III, respectively. ORF I contains p8, p12, and p27 sequences, while ORF II codes p30 and p13. HTLV-1 bZIP factor (HBZ) is an anti-sense gene that is coded for in the complimentary minus RNA strand (D'Agostino *et al*., 2019).

The genome is flanked by two long terminal repeats (LTR) sequences that are each composed of a unique 3' (U3'), a repeating region (R), and a unique 5' (U5') region that are responsible for the initiation of RNA synthesis and transcription, splicing, polyadenylation, and reverse transcription strand transfer (Ratner, 2020; Hirons *et al*., 2021) as shown in figure (2.1). (Barbeau *et al*., 2013)

The life cycle of HTLV-1 involves several key steps that are unique to retroviruses, including reverse transcription and integration into the host genome. In the first step of infecting a cell, HTLV-1 binds to target cell plasma membrane surface receptors, including Glut-1, neuropilin-1 (NRP-1), and heparin sulfate proteoglycans (HSPG) (Zhang *et al*., 2017; Eusebio-Ponce *et al*., 2019).

The virus envelope fuses with the host cell membrane following binding, and the viral core is released into the cytoplasm. Once inside the cell, the RT enzyme converts the single-stranded RNA genome to a double-stranded DNA form by initiating host-derived tRNA. The newly created double-stranded DNA intermediate is next transported into the nucleus for integration as part of a pre-integration complex containing CA, RT, IN, and NC proteins as shown in figure (2.2) (Zhang *et al*., 2017; Raza *et al*., 2021).

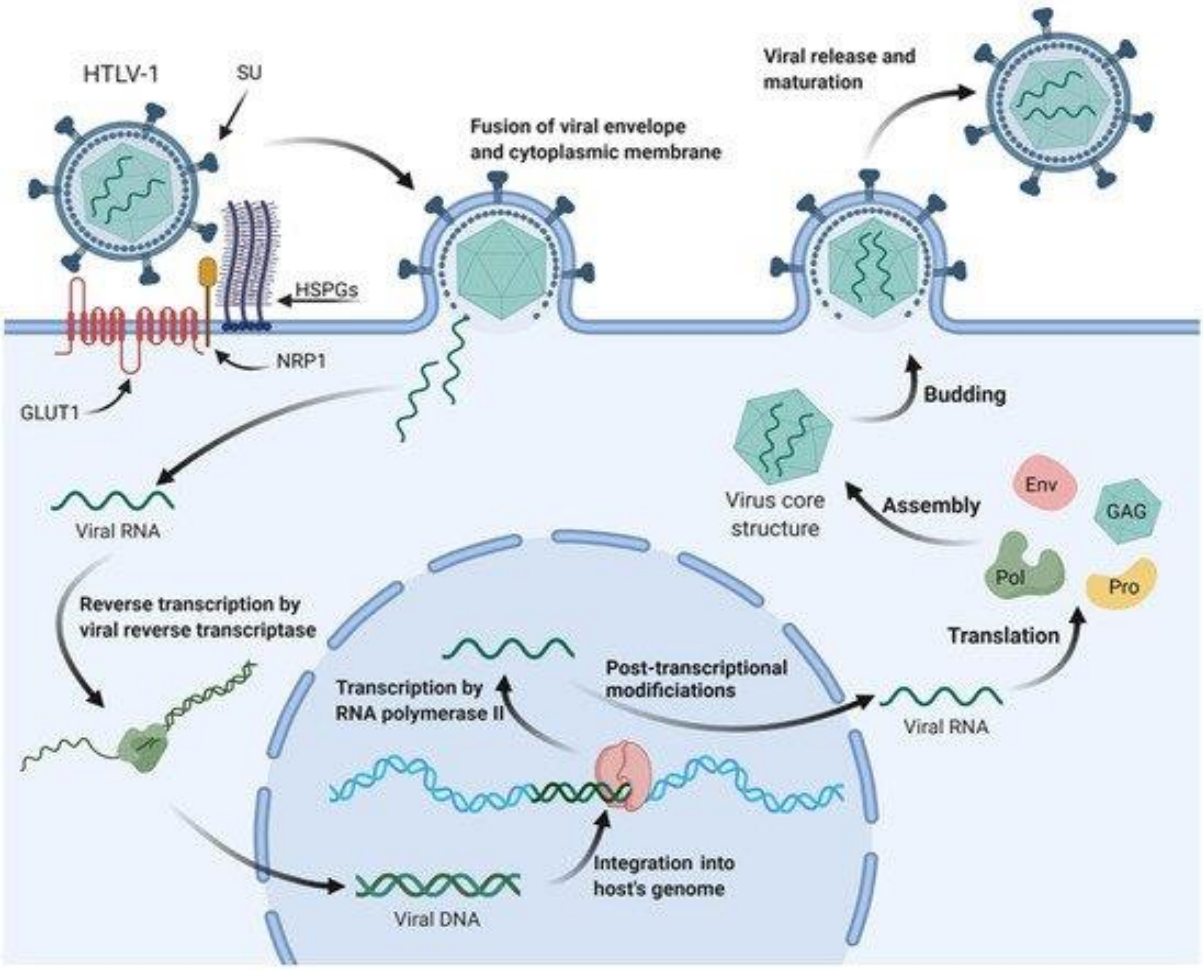

Figure (2.2). HTLV-1 Life Cycle (Machado, *et al.*, 2022).

2.2.4. Structural and enzymatic proteins of *HTLV-1*

Human T-cell lymphotropic virus type-1 has developed multiple strategies for coding a large amount of information into a relatively small genome. Full-length RNA transcripts are utilized for replication purposes. Still, alternative splicing, ribosomal frameshifts, and post-translational modifications of nascent proteins are all used to ultimately generate the various proteins needed to complete the virus replication cycle (Martin, 2016).

The structural and enzymatic proteins of *HTLV-1* are no exception. They are generated from a Gag/Pro/Pol precursor protein processed and post-translationally modified within the cytoplasm to form the final proteins for viral progeny (Thulson *et al*., 2020).

The gag is post-translationally myristylated in the host cell cytoplasm and cleaved by viral protease into its three functional proteins during viral budding to form a mature, infectious viral particle. At the center of the viral particle, the nucleocapsid interacts with the full-length genomic RNA transcripts. Capsid proteins then surround this core and, outside that, matrix proteins. The p55 Gag polyprotein contains the structural elements capsid (CA; p24), nucleocapsid (NC; p15), and matrix (MA; p19). The matrix is needed for viral budding and assembly utilizing its PPPY domains and appears to assist in cell-to-cell transmission (Mattei *et al*., 2016).

The viral envelope is the outermost layer of the virus particle (Mohanty and Harhaj, 2020). The envelope glycoproteins of *HTLV-1* are composed of 288 amino acids with 4 N-glycosylation sites and an apparent mass of 46 kDa (gp46). Surface (SU) is associated, most likely via labile disulfide bonds, to the membrane-anchored TM (Transmembrane), which is 180 amino acids long with one N-glycosylation site and an apparent mass of 21 kDa (gp21) which is subsequently translated on ribosomes in the endoplasmic reticulum (Maksimova *et al*., 2022).

The Env precursor passes through the Golgi apparatus to the Tran Golgi network, cleaving it by a cellular protease of the furin family into SU and TM subunits. SU-TM complexes organized as trimers are then transported to the surface of infected cells, where incorporation into the budding particles occurs (Hogan and Johnson, 2023).

Both subunits form heterodimers at the cell surface and are required for viral infectivity. The SU interacts with host cell receptors to initiate viral entry. Following binding to heparan sulfate proteoglycan (HSPG) and neuropilin 1(NRP-1), there is a conformational change in SU that allows for interactions with Glucose transporter 1(GLUT-1), resulting in the TM-mediated cell fusion of the cell membrane and viral envelope (Gross *et al*., 2016; Martin *et al*., 2016).

The enzymatic elements of HTLV-1 formed by the Pol precursor polyprotein are integrase (IN), reverse transcriptase (RT), and protease (Pro). Reverse transcriptase and IN are translated together from the bicistronic RNA that contains Gag through the use of a single ribosomal frameshift to access an alternate open reading frame. Protease is generated from the same Gag/Pol RNA after two ribosomal frameshifts (Kassay *et al*., 2021; Park *et al*., 2022).

Protease is initially generated in an inactive form. After viral budding, it self-cleaves to its active form and allows for the maturation of the viral particle by cleaving Gag and Pol polyproteins into their final products (Haines, 2012; Kassay *et al*., 2021).

2.2.5. Regulatory proteins of HTLV-1

2.2.5.1. Transactivator protein X (Tax)

Tax is the transcriptional activator of the PX region. Tax is a 353 amino acid, 40 kDa doubly spliced phosphoprotein with nuclear localization encoded in the open reading frame (ORF) IV (Bangham, 2018). It exerts most of its activity in the nucleus, which regulates viral transcription. Still, it has been shown to translocate to the cytoplasm using a nuclear export signal protein sequence (Nozuma e al., 2020). Tax acts to cooperatively bridge cellular proteins through the indirect

binding of viral DNA at the LTR in Tax responsive elements (TREs) (Mohanty and Harhaj, 2020).

There are three TREs (TRE-1, TRE-2, TRE-3) present in the U3 region of the LTR. TRE-1 contains GC-rich regions, which share homology with the cellular CRE (cAMP 8 responsive element). Tax uses a similar mechanism to the host cell by binding and stabilizing CREB/ATF transcription factors to the TRE for transcription initiation (Watanabe, 2017; Ratner *et al*., 2020).

Tax's ability to transform lymphocytes is likely related to its ability to influence the expression of genes involved in apoptosis and DNA repair, such as Bcl-x, Bax, and proliferating cell nuclear antigen (PCNA) (de Oliveira Andrade *et al*., 2021). The tax has been reported to be involved in viral transmission by promoting cell adhesion and developing the virological synapse between infected and target cells. One method that Tax appears to act is through increased expression of (C-C motif chemokine 22 is a protein that in humans is encoded by the CCL22 gene) CCL22a ligand for the CD4+ lymphocyte receptor CCR4 (Shafiei and Mozhgani, 2023). Tax has also been shown to enhance the expression of intercellular adhesion molecule 1(ICAM-1) on the surface of infected cells, which leads to the formation of the virological synapse (Fazio *et al*., 2019). Both pathways may be important Tax mediated mechanisms for attracting target cells (Haines, 2012).

2.2.5.2. Regulator X (Rex)

Rex is 27 kD, a phosphoprotein encoded in ORF III that contains a nuclear localizing sequence (NLS), a nuclear export sequence (NES), an RNA binding domain, and a multimerization domain (Malu *et al.*, 2019). The NES allows the Rex/mRNA complex to translocate from the nucleus into the cytoplasm (D'Agostino, 2019; Lyngdoh *et al*., 2019). Rex is primarily involved in post-

transcriptional modification and regulation of viral DNA. It helps to transport unspliced DNA from the nucleus (gag, pol, env) by binding to a Rex response element (RxRE) in the viral RNA. The RxRE is present within the U3/R region of the 3' LTR (Nakano *et al*., 2022).

Rex can also interfere with the splice to inhibit viral RNA splicing and can help stabilize doubly spliced viral mRNA by retaining it in the nucleus. Rex is not required for immortalization in vitro but for optimal viral transmission, as confirmed in the rabbit model using molecular clones with a Rex-deficient mutation (Nakano and Watanabe, 2016).

2.2.6. Non-structural proteins of HTLV-1

The non-structural proteins of HTLV-1 are located in ORF I and II within the pX region (Fukumoto, 2013; Lyngdoh *et al.,* 2019). Four proteins are produced through alternate splicing, and all contain a common 1st non-coding exon from the R region of the 5' LTR. Multiple splice acceptor sites result in either singly (p12 and p13) or doubly spliced products (p27 and p30) (Bangham and Matsuoka, 2017).

The proteins within this region were initially known as accessory proteins because they were not believed to be essential for viral transmission in vitro (Afonso *et al.,* 2019). It is now known that these proteins play a critical role in viral infectivity, maintenance of proviral loads, and host-cell interactions for viral and host-cell gene transcription (Barreto *et al.,* 2016).

2.2.6.1. HTLV-1 basic leucine zipper factor (HBZ)

HTLV-1 basic leucine zipper factor (HBZ) is a unique 209 amino acid, 31-kDa protein encoded on the antisense strand that overlaps with the ORF for p12, p30, and p13. Similar to p30, it appears that HBZ acts antagonistically to Tax. It

contains three main motifs, a nuclear localization motif, an N-terminus activation domain, and a C-terminus leucine zipper motif, which assist in the protein's functions as a transcription factor (Shallak et *al*., 2021). It is expressed as three mRNA products, with a major spliced variant (sHBz) being the most dominantly expressed (Malu, 2019).

HBZ anti-Tax activity occurs mainly through indirect DNA binding. One method that accomplishes this is through inhibition of the recruitment of CBP/p300 by Tax for viral transcription. Similarly, HBZ forms dimmers with CREB-2, c-Jun, AP-1, and JunB, which can interfere with Tax recruitment, DNA binding, and viral transcription (Matsuoka and Mesnard, 2020; Nozuma *e al*., 2020).

HBZ binding does not always result in down-regulation of transcriptional activity. In some instances, JunD activity can be increased through HBZ interactions, which may lead to tumor progression in ATL patients (Ahmadi *et al*., 2023).

In regulating genomic integrity, T-cell proliferation, inhibits apoptosis and autophagy in HTLV-1-infected cells (Tanaka *et al*., 2014; Ma *et al*., 2016). Among all the viral proteins, experimental evidence implicates Tax as the viral oncoprotein, but emerging data suggest a supporting role for HBZ in the oncogenic process (Hleihel *et al.,* 2023). Deletion of HBZ results in decreased antibody response and pro-viral load in the rabbit model and reduced tumor growth in the mouse model of ATL (Yasir Khan *et al*., 2017).

2.2.7. Viral transmission

Of the many possible routes of virus transmission, mother-to-child through breastfeeding is the most predominant mode (Percher *et al.,* 2016). Sexual

transmission rates are 60% for male to female but only 0.4% for female-to-male transmission (Cassar and Gessain, 2017).

Predisposing factors associated with sexual transmission include the presence of genital ulcers, high viral loads, and high antibody titers in the donor. Among non-drug-using sexual partners of injecting-drug users IDUs, sexual transmission is a more common mode than parenteral transmission (Motomura *et al*., 2019; Bryan and Tadi, 2021).

Among IDUs, blood, and blood products are the most significant source of infection. Approximately 12% of HTLV infections occur by blood transfusion. Unlike HIV-1, whole-cell transfusion is required for transmission of the virus; however, the risk of transmission decreases markedly if the blood units are stored for more than six days before transfusion (Goto *et al*., 2020; Pise-Masison and Franchini, 2022).

As with HIV-1 infection, dendritic cells (DCs) have been demonstrated to play a biphasic role in cell-to-cell transmission of HTLV-1. DCs can capture and transfer the virions to fresh T cells in a trans fashion or transmit de novo synthesized virions upon infection to new T cells in a cis fashion (Shimauchi *et al*., 2019).

2.2.8. Epidemiology of *Human T- Lymphotropic Virus Type 1* (*HTLV-1*)

Worldwide, approximately 15-20 million people are infected by the virus (Araujo and Wedemann, 2019; Legrand *et al*., 2022). *Human T-cell lymphotropic virus type 1* is endemic to Japan, central Africa, Central and South America, the Caribbean basin, islands in the Pacific basin, and within the aboriginal population of Australia (Willems *et al*., 2017; Ito *et al*., 2021). Within endemic regions, the seroprevalence is highly variable, ranging from 0.1 to up to 30% (Gessain *et al*., 2023).

Disease development is sporadic among seropositive populations, affecting 1 to 5% of infected individuals. These individuals primarily will develop ATL or HAM/TSP, with an even smaller percentage developing HTLV-1-associated inflammatory diseases, including uveitis, polymyositis, arthritis, and dermatitis (Rosa *et al*., 2022).

A study on HTLV-1 carriers in Japan showed that the estimated number of HTLV-1 carriers in 2007 was one million and that HTLV-1 carriers have spread to other geographical areas in Japan. Therefore, in Japan, since 2011, the nationwide screening of pregnant women for HTLV-1 infection has been recommended to prevent breast milk-borne transmission of HTLV-1 (Miura and Masuzaki, 2017).

2.2.9. Pathophysiology of HTLV-1

The attachment and fusion of the virus to the target cell. It is estimated that HTLV-1 persists in approximately 10^3–10^6 infected T-cell clones within an infected host. Initial infection requires crossing the mucosal barrier and then infection of mucosal immune cells directly or via Antigen presenting cells (APCs). The virus could cross the mucosal barrier through various mechanisms (Carpentier *et al*., 2015):

1. Transmigration of HTLV-1 infected macrophages: Infected macrophages transmigrate through the epithelium during breastfeeding and sexual intercourse.
2. Transcytosis of viral particles: In this process, a virion is incorporated into a vesicle and is transferred from the apical to the basal surface of an epithelial cell.

3. Release of newly produced virions from the basal surface of an infected epithelial cell: HTLV-1 infects an epithelial cell and produces new virions that are then released through the basal surface.
4. Bypass of HTLV-1 infected cells through a damaged mucosa: Infected cells can enter where mucosa integrity is damaged.
5. Viruses penetrate the microflora cells in the gut lumen and then infect macrophage and epithelial cells.

After initial viral infection, the virus is thought to become transcriptionally silent or latent, as it is difficult to detect and sense viral transcripts or proteins in infected individuals. The viral RNA is delivered into the cytoplasm and is converted into double-stranded DNA (dsDNA) through reverse transcription, and then dsDNA is integrated into the host nuclear genome. The integrated form (proviral DNA) contains direct sequence repeats that flank the internal coding region and are known as long terminal repeats (LTRs) (Bangham *et al*., 2019).

All retroviruses have three major genes, arranged in the order 5-gagpol-env-3, and can be transmitted horizontally from cell to cell. The gag gene encodes structural proteins, including those forming the viral capsid, the pol gene encodes enzymatic activities needed for virus propagation (i.e., protease (PR), RT, and integrase (IN)), and env contains the genetic information necessary for expression of proteins mediating infection and viral entry (Menéndez-Arias *et al*., 2017). However, the presence of activated cytotoxic T lymphocytes (CTLs) directed against sense viral antigens would suggest that some viral transcription is present in HTLV-1-infected individuals (Billman *et al*., 2017).

Cellular RNA polymerase II transcribes this provirus; subsequent posttranscriptional regulation is essential for splicing and transporting HTLV-1 mRNA. Then, the viral mRNA is exported from the nucleus to the cytoplasm, and viral proteins are translated and transported to the plasma membrane with two copies of genome RNA that form a virus particle at the virus budding site of the plasma membrane. These budding particles are released from the cell surface, undergoing a maturation process by the action of viral proteases (Martin, 2016).

Tax and HBZ play a crucial role in the cell alteration process by triggering changes in various intracellular signal transduction pathways, both by up-regulating and down-regulating viral and cellular gene expression, to initiate neoplastic transformation. HTLV-1 Tax, once it is produced, forms a complex with the transcription factors on the 5′ LTR and recruits CBP/p300, thereby enhancing viral gene transcription (Wang *et al*., 2020). Regulation of HTLV-1 gene expression allows the virus to evade immune detection, immortalize infected target cells, and establish persistent infection (Tu *et al*., 2022).

The accessory genes *p12*, *p13*, and *p30* play significant roles in establishing and maintaining viral persistence. In contrast, Rex regulates post-transcriptional viral gene expression and increases the stability of viral RNA for the latency phase of the viral life cycle (D'Agostino *et al*., 2019).

2.2.10. HTLV-1 and Brain Disease

The central nervous system (CNS) was considered an immune-privileged site with no lymphatic drainage. Still, it is now recognized to mount robust immune responses to various CNS viral infections that are unique from the immune response in peripheral tissues. The different outcomes of an HTLV-1 infection are

thought to be associated with differences in the host response to the virus rather than the virus itself (Enose-Akahata *et al*., 2019).

The ability of HTLV-1 to cause associated neuropathies starts with the virus crossing the blood-brain barrier (BBB), then entering and infecting the cells of the central nervous system. Consequently, to the viral attack, HTLV-1 infected lymphocytes produce pro-inflammatory cytokines like tumor necrosis factor-alpha, Interleukin 1 beta, and interleukin 6, further disrupting the BBB (Yasir Khan et *al*., 2017).

The progression from HTLV-1 infection to disease is a consequence of HTLV-1 replication in CD4+ T and CD8+ T lymphocytes and the imbalance between proinflammatory and anti-inflammatory cytokines (Brites *et al*., 2021).

HTLV-1-infected T cells and CD8+ cytotoxic T lymphocytes (CTL) against HTLV-1 invade the central nervous system (CNS) and release proinflammatory cytokines and chemokines, resulting in tissue damage. HTLV-1 viral regulatory proteins, Tax, and HTLV-1 basic leucine zipper factor (HBZ) play critical roles in immune dysregulation in HTLV-1 infection (Crawshaw *et al*., 2018).

The CNS is protected from the entry of pathogens, circulating immune cells, and factors within the blood by a physiological structure called the blood–brain barrier (BBB), which is maintained by the endothelial cells of cerebral microvessels with tight junctions. CD4+ T cells are routinely exposed in vivo to alterations in the microenvironment, which was associated with enhanced production of various soluble factors as well as expression of cell adhesion molecules due to activation of HTLV-1 expression (Manivannan *et al*., 2016).

2.2.11. Laboratory Diagnosis of HTLV-1

1- Serology

Serological screening for *human T-cell lymphotropic virus type 1* (HTLV-1) is usually performed using enzyme-linked immunosorbent assay (ELISA), Chemiluminescent microparticle immunoassay (CMIA) (Hedayati *et al*., 2022).

2- Qualitative Duplex Real-Time PCR Method

Rapid, high sensitivity and specificity method for discriminatory and confirmatory diagnosis of HTLV-1 Infections (Kashima *et al*., 2022).

3- Qualitative TaqMan Real-Time PCR Method

A quick and specific technique to detect the quantity of target virus via the inlet fluorescence probes specifically allows the detection of proliferative products directly related to the number of target copies (Hamidi *et al*., 2018).

4- Qualitative PCR Method

Quantitative PCR (qPCR) analysis of human T-cell leukemia virus type 1 (HTLV-1) was used to assess the amount of HTLV-1 provirus DNA integrated into the genomic DNA of host blood cells (Kuramitsu *et al.*, 2015).

2.3. Tumor Necrosis Factor Alpha (TNF-α)

TNF-α is a potent pro-inflammatory cytokine exerting pleiotropic effects on various cell types. It acts as a central biological regulator in critical immune functions and is critical in the pathogenesis of chronic inflammatory diseases (Zia *et al*., 2020).

TNF-α is encoded by a single copy gene located in human chromosome 6p21.3 between the HLA-B and HLA-DR genes and is closely located to the genes of the major histocompatibility complex (El-Tahan *et al*., 2016).

TNF-α is a homotrimer protein consisting of 157 amino acids which exists in a soluble and transmembrane form. The members of the TNF-α family exert their effects through two distinct receptors, TNFRSF1A (TNFR1) and TNFRSF1B (TNFR2). TNFR1 is ubiquitously expressed and found in all cell types, while TNFR2 is mainly found on immune cells, mostly expressed in restricted cell subtypes like neurons, oligodendrocytes, astrocytes, endothelial cells, and subpopulations of T lymphocytes, among others where its pathway activation by TNF-α assists in regulating the immune response and inflammation (Jang *et al*., 2021)

TNF-α has both tumor-promoting and tumor-suppressing roles in the tumor microenvironment; the tumor cell continuously produces endogenous TNF-α, which induces tumor angiogenesis and promotes progression. TNF-α plays an important role in tumor metastasis. In the central nervous system (CNS), the term neuroinflammation is used to denote cellular and inflammatory responses of vascularized neuronal tissue through activation of resident cells in the brain (microglia, astrocytes, and endothelial cells), the recruitment of blood-derived leukocytes, including neutrophils, lymphocytes, and macrophages, and a plethora of humoral factors (Muhammad, 2019; Laha *et al*., 2021).

I. TNF-α Gene Polymorphism in Brain Tumors

Single-nucleotide polymorphisms (SNP) within TNF-α can potentially affect the function or regulation of TNF-α production. Several SNPs have been identified in its promoter. The TNF-α-308G/A (rs1800629), -238G/A (rs361525), and -863C/A(rs1800630) in the promoter region of the TNF-α gene are 3 common functional polymorphisms that have been demonstrated to be associated with the production level of the cytokine (Ding *et al*., 2014).

Various studies have shown that polymorphisms at position -308 and -863 in the promoter region of the TNF-α gene were associated with risks of subarachnoid hemorrhage (SAH) in multiple ethnicities (Yamada *et al*., 2006; Fontanella *et al*., 2007).

The TNF-α-1031T/C (rs1799964), -857C/T (rs1799724) are newly discovered gene polymorphisms that have affected periodontitis susceptibility (Barnea *et al*., 2015; Majumder *et al*., 2018).

Kumar *et al*. (2020) have reported four polymorphic regions (-308G/A, +488G/A, -857C/T, and -1031T/C) in the promoter region of the TNF-α gene associated with Intracerebral Hemorrhage in the north Indian population.

In another study, TNF-α gene is considered a cause of the generation of neurological diseases in brain tissue produced by astrocytes (Singh *et al*., 2021).

2.4. Interferon-gamma (IFN-γ)

IFN-γ is a cytokine involved in the induction and modulation of various immune responses. The main producers of IFN-γ include CD4 Th1 cells, CD8 T-cells, NK cells and NKT cells (Kak *et al*., 2018).

Gene encoding IFN-γ protein is located on chromosome 12q15 in humans, and in the mouse genome, it is found on chromosome 10D2. The human gene for IFN-γ consists of four exons and three intervening regions, covering 5.4 kb (Bhat *et al*., 2018).

IFN-γ is a protein encoded by the *IFN-γ* gene, composed of two polypeptide chains associated in an antiparallel fashion (Jorgovanovic *et al*., 2020). IFN-γ is a homodimer consisting of non-covalent self-assembly in a head-to-tail orientation. The helical regions A and B with their connecting loop, a histidine residue at

position 111 (H_{111}) in the F helix, and the flexible C terminus are important regions for receptor binding. Ligand binding results in receptor oligomerization, with two α-receptor chains, IFN-γR1, bound to one IFN-γ homodimer, followed by recruitment of two β-receptor chains, IFN-γR2, to the complex inducing the expression of IFN-γ-stimulated genes (Mendoza *et al*., 2019).

Interferon-gamma (IFN-γ) is one of the critical factors of both innate and adaptive immune response. That promotes the differentiation of naive CD4+ cells into effector Th1 T cells producing the main mediators of cellular immunity against viral; intracellular bacterial infections; protozoa infection; specific cytotoxic immunity through the interaction of T cells with antigen-presenting cells and macrophage activation, increased expression of MHC molecules, antigen-processing components, Ig class switching and suppress the cells (Rakityanskaya *et al.,* 2022).

IFN-γ is considered potentially useful for adjuvant immunotherapy for different types of cancer through stimulation of antitumor immune response. IFN-γ acts as a cytotoxic cytokine together with granzyme B and perforin to initiate apoptosis in tumor cells (Maimela *et al*., 2018) but also enables the synthesis of immune checkpoint inhibitory molecules and indoleamine-2,3-dioxygenase (IDO), thus stimulating other immune-suppressive mechanisms (Mojic *et al*., 2018; Jorgovanovic *et al*., 2020).

I. IFN-γ Gene Polymorphism in Brain Tumors

A single nucleotide polymorphism (SNP) in the first intron of the human IFN-γ gene containing a nuclear factor-$_K$B (NF_KB) -binding region is associated with several autoimmune and inflammatory diseases (Nayeri *et al*., 2019).

Nayeri *et al*. (2019) studied the IFN-γ +874 T/A (rs2430561) gene polymorphism in 106 BD patients and 109 control subjects using sequence-specific primers (SSPs) or amplification refractory mutation system-polymerase chain reaction (ARMS-PCR).

The T allele is high, while the A allele is low, plasma IFN-γ. Although prior studies illustrated the dysregulation of serum IFN-γ in B1D, few studies have addressed IFN-γ polymorphisms, and their conclusions have been contradictory (Clerici *et al*., 2009; Yoon *et al*., 2012).

2.5. Killer Immunoglobulin-like Receptors (KIRs)

KIRs are cell surface molecules expressed on NK cells. They were initially defined as inhibitory receptors and named 'Killer-cell Inhibitory Receptors'. When activating receptors within this family, both groups were named 'killer-cell immunoglobulin-like receptors (KIRs) (Dębska *et al*., 2021).

The KIR receptors are encoded by a set of 15 genes for inhibitory and activating receptors known as the leukocyte receptor complex (LRC) on chromosome 19q13.4 (Dębska *et al*., 2021).

KIRs are a family of membrane glycoproteins expressed by NK cells. KIRs contain two or three extracellular immunoglobulin-like domain molecules (D) with a long (L) or short (S) cytoplasmic tail (Paul and Lal, 2017).

KIR genes exist as several allelic forms, which makes them the most polymorphic human family of NK cell receptors. The KIR gene family consists of 15 gene loci (KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR3DL2, KIR3DL3, KIR2DS1, KIR3DL1/S1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5) and 2 pseudogenes (KIR2DP1 and KIR3DP1) (Dębska *et al.*, 2021).

KIR molecules are grouped on NK cells into stimulatory and inhibitory KIR haplotypes A and B, through which NKs sense and tolerate HLA self-antigens or up-regulate the NK-cytotoxic response to cells with altered HLA self-antigens, damaged by viruses or tumors. Upon engagement with HLA class I molecules, KIRs block NK cell activation and function (Dębska *et al*., 2021)

Because the recognition of HLA by KIR modulates NK function and these cells are important for attacking tumors, variations in KIR and HLA have been thought to interfere intensely with the risk of developing cancer (Augusto, 2016).

Inhibitory killer cell immunoglobulin-like receptors (iKIRs), which are expressed on natural killer (NK) cells as well as CD8+ T-cells. iKIR enhances the HLA protective and detrimental effects through binding with their HLA class 1 ligand and increasing the lifespan of CD8+ T-cells (Boelen *et al*., 2018).

I. KIR Gene Polymorphism in Brain Tumors

Genotypic KIR diversity is a result of allelic and haplotypic diversity. Thus, detecting the same KIR genotype in two unrelated individuals is extremely difficult. Due to these characteristics, KIR genes are considered good markers of determining population genetics (Sarac *et al*., 2019).

Two different haplotypes of KIR genes have been reported. The A haplotype with an activating KIR gene, KIR2DS4, and five inhibitory KIR genes (KIR2DL1, KIR2DL3, KIR3DL1, KIR3DL2, and KIR3DL3), whereas the B haplotype has a variable number of activating and inhibitory genes. KIRs act by binding to specific alleles of HLA-C, HLA-B, or HLA-A (Middleton *et al*., 2014).

Gras Navarro *et al*. (2014) dictate that the KIR2DS2 gene may identify all reactive NK cell subsets with higher potency against glioblastoma (GBM), independently of the repertoire of inhibitory or other activating KIRs. KIR2DS4+

NK cells were more potent against GBM than KIR2DS22/KIR2DS42 NK cells but were not significantly more potent than KIR2DS2+ NK cells. That indicates that an intrinsic cytotoxic potency, rather than KIR–HLA ligand interactions, is the mechanism of potency associated with the KIR2DS2 immunophenotype.

Furthermore, Tuttolomondo *et al*. (2018) show an association between some KIR genes and HLA-ligand alleles and susceptibility to develop symptomatic acute viral encephalitis.

2.6. Interleukin 1 receptor antagonist (IL-1Ra)

IL-1Ra is a receptor antagonist related to the IL-1 family that binds in an inactive fashion to the same receptor as IL-1β and IL-1α, thereby antagonizing them (Boraschi, 2022). IL1Ra encoding gene is found in chromosome 2q14 (Pehlivan *et al*., 2022).

IL-1RA gene has a penta-allelic polymorphic region that contains variable numbers of an 86-bp tandem repeat (VNTR) (rs2234663) in intron 2 (Pehlivan *et al*., 2022).

The interleukin-1-receptor antagonist IL1RA (encoded by the *IL1RN* gene) is a potent competitive antagonist to interleukin-1 (IL1) without inducing signal transduction; and thereby is mainly involved in the regulation of inflammation (Schneider *et al*., 2021).

IL-1 receptor antagonist has been identified in neurons that can block IL-1β pro-inflammatory actions by competing with IL-1β for IL-1R binding. Thus, IL1RA exerts potent anti-inflammatory and neuroprotective actions in the brain and other tissues (Sun *et al.*, 2017).

I. IL-1Ra Gene Polymorphism in Brain Tumors

The classical *IL-1RN* gene has four exons; currently, about 140 SNPs have been recorded for this gene. In addition, the *IL-1RA* gene has a penta-allelic polymorphic region that contains variable numbers of an 86-bp tandem repeat (VNTR) (rs2234663) in intron 2 (Khazim *et al*., 2018; Pehlivan *et al*., 2022).

IL-1Ra can cross the blood-brain barrier; thus, protein measured in the brain may be of peripheral or local origin (Atteq *et al*., 2023).

Hadjigeorgiou *et al*. (2005) reported that the presence of IL-1RN allele 2 is associated with brain hemorrhage.

The specific SNPs in IL1RA can indicate clinical outcomes for some groups of patients with metastatic breast cancer being treated with chemotherapy (Lafrenie *et al*., 2023).

2.7. Interleukin 28 (IL-28)

IL-28, also called IFN-λ2/3, is a newly identified member of the IL-10-interferon cytokine family. *IL28A* belongs to Type III IFN (IFN-λ), a group of cytokines related to Type I IFN and eliciting similar antiviral effects. IL-28A is mainly produced by dendritic cells following viral and bacterial infection and mediates its effect through interaction with the IL-28R/IL-10R2 receptor complex (Xu *et al*., 2021). IL28A is located on the long arm of chromosome 19 (19q13.13 region) (Syedbasha and Egli, 2017).

Four IFN-λ subtypes have been found in humans: IFN-λ1 (IL-29), IFN-λ2 (IL-28A), IFN-λ3 (IL-28B), and IFN-λ4. All of these proteins are encoded on the 19th chromosome, and these genes consist of five or six exons (Lozhkov *et al*., 2020).

The indirect mechanism of IFNλ regulation of T cell responses could yield exciting insights into the ability of IFNλ to be utilized as a therapeutic or vaccine adjuvant to augment the immune response against viral infections in collaboration with other IFNs (Hemann *et al*., 2017).

I. IL-28 Gene Polymorphism in Brain Tumors

IL28 (also known as interferon lambda) IFN-λ tightened the blood-brain barrier (BBB), restricted viral neuroinvasion, reduced viral titers in the CNS, and protected mice from lethal viral infection (Lazear *et al*., 2015).

IL28 polymorphisms are more likely to occur among *HTLV-1*-infected subjects, and IL28 polymorphisms are associated with higher proviral loads in *HTLV-1* carriers (Treviño *et al*., 2012).

2.8. Diagnostic of TNF-α; IFN-γ; KIR; IL-1Ra and IL-28 Polymorphism

1. Single Strand Conformation Polymorphism (SSCP)

SSCP analysis is a simple and sensitive technique for mutation detection and genotyping. The principle of SSCP analysis is based on the fact that single-stranded DNA has a defined conformation. Altered conformation due to a single base change in the sequence can cause single-stranded DNA to migrate differently under no denaturing electrophoresis conditions (Darwish *et al.,* 2022).

2. Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is a technique that exploits variations in homologous DNA sequences, known as polymorphisms, in order to distinguish individuals, populations, or species or pinpoint the locations of genes within a sequence. The term may refer to a polymorphism itself, as detected

through the differing locations of restriction enzyme sites, or to a related laboratory technique by which such differences can be illustrated. In RFLP analysis, a DNA sample is digested into fragments by one or more restriction enzymes, and the resulting restriction fragments are then separated by gel electrophoresis according to their size (Chaudhary and Kumar, 2020).

3. Amplification Refractory Mutation System (ARMS)

In the ARMS technique, one PCR comprises one allele-specific oligonucleotide primer at 5′-end and a common primer at 3′-end. If the presence of an amplified mutant is detected by agarose gel electrophoresis, it suggests that the target sequence contains the mutant allele. Similarly, if the result displays an absence of the amplified mutant, it indicates the presence of the normal DNA sequence on that specific point. In the same way, a normal primer at 5′-end together with a common primer at 3′-end was used in another PCR. If the normal amplified product is present, it reveals the existence of a natural DNA sequence, whereas if the normal amplified product is absent, then it reveals the presence of a mutant allele (Yang *et al.,* 2017).

4. Sanger Sequencing Methods

Sanger sequencing has been the gold standard for many years. The sequencing reaction is read as a color code that distinguishes oligonucleotides of variable length with four specific color labels. A ladder of nucleotides is created that can be identified by their electrophoretic mobility and the color of terminal fluorescent nucleotides. Computer programs that not only create the chromatogram but also compare them with reference sequences and identify abnormalities that represent mutations and polymorphisms can facilitate the identification of abnormal (mutant)

peaks in chromatograms of this ladder. Sanger sequencing is readily accomplished in DNA extracted from blood and other tissue that has been amplified by PCR provided that the amplified sequence is short (<300 base pair). This method has the advantage that it provides unbiased sequence results that will detect virtually any mutation in the targeted region. However, the analytic sensitivity is limited, with a tumor cell concentration of approximately 50% required for accurate results. Because of the PCR amplification, the amount of DNA starting material required is usually small, depending on the number of DNA templates selected for amplification (Huseynova, and Ansarova, 2023).

5. Sequencing of PCR Products

The term DNA sequencing refers to methods for determining the order of the nucleotide bases adenine, guanine, cytosine, and thymine in a molecule of DNA.

Academic researchers, using laboratory methods based on 2- dimensional chromatography in the early 1970s, obtained the first DNA sequence. With the development of dye-based sequencing methods with automated analysis, DNA sequencing has become easier and faster. The knowledge of DNA sequences of genes and other parts of the genome of organisms has become indispensable for basic research studying biological processes, as well as in applied fields such as diagnostic or forensic research (Booth *et al.,* 2015).

Chapter Three

Materials and Methods

3. Materials and Methods

3.1. Subjects

3.1.1 Patients population

This cross sectional case-control study was done for one-hundred-fifty specimens collected from brain tissues as well as blood specimens. The studied tissues of brain tumors obtained from patients were related to those aged 2 years to 85 years. While the collected autopsies from the non-brain tumors control cases who have, aged 16 years to 71 years were obtained from dead cases for non-neurological causes who have shown normal brain histology. The specimens were collected during the period from January to September (2022).

Brain tissues as well as Blood from patients with brain tumors should be enrolled and classified into:-.

1. Seventy -five brain tissues as well as blood specimens from patients with brain tumors.
2. Twenty -five brain tissue autopsies from the non-brain tumors as a control group.
3. Fifty blood specimens from persons as apparently healthy control groups.

All these specimens were submitted for the viral genome extraction; total DNA genome extraction as well as separation of blood serum were obtained and stored at -70°C till used. The first part of the work was done for *HTLV-1* detection by Real-time- PCR technique, while, the second part was done for estimation of TNF-α, IFN-γ, KIRs, IL1Ra, and IL-28 genes polymorphisms by sanger sequencing. Another routine was used to evaluate the levels of TNF-α, IFN-γ, KIRs, IL1Ra, and IL-28 in patients with brain tumors and apparently healthy control groups by ELISA technique.

3.2. Study Design

Figure (3.1) show the design of currently study

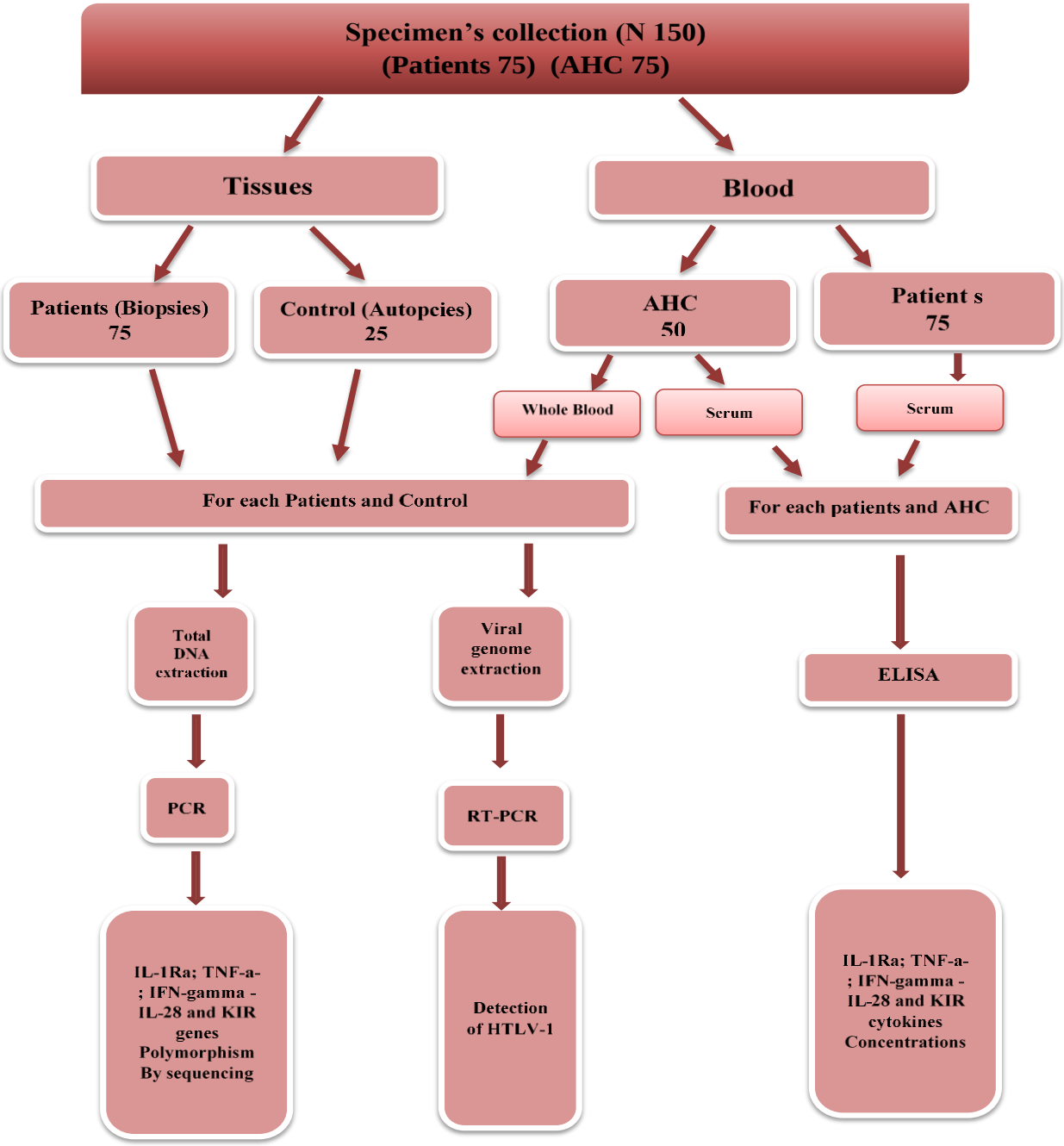

Figure (3.1): Study Design

3.3. Materials

3.3.1. Instruments and Equipment

The instruments and equipment used in this study are listed in table (3-1 A-B).

Table 3.1.A: Instruments used in this study with their Manufacturer Company and origin

Instruments	Manufactured Company (Origin)
Autoclave	Hirayama
Centrifuge	Hettichzentrifuge, Sigma / Germany
Cool centrifuge	Hettichzentrifuge, Sigma / Germany
Deep freeze (-20 C)	Bosch / Germany
Electrophoresis system	Germany
ELISA reader	Bio Tech (USA)
Fume hood	UK
Hot plate	Memmert / Germany
Incubator (model IB-909)	Memmert / Germany
Microcentrifuge	Hettichzentrifuge, Sigma / Germany
Multibiodrop	England
Real time Qiagen device Rotor Gene Q MDx	QIAGEN / Germany
Real time soft wear system	QIAGEN / Germany
Sensitive balance	Sartoris / Germany
Water bath (1-A)	Memmert / Germany

Table 3.1.B: Equipment used in this study with their Manufacturer Company and origin.

Equipment	Manufactured Company (Origin)
0.2ml PCR Tubes with Flat Caps	Extragene / Taiwan
1.5ml clear Microtubes	
Disposable plastic container	China
Disposable tips	Extragene/Taiwan
Eppendorf tubes with different size 2ml, 1.5ml, 0.5 ml	
Falcon with different size 15ml , 50 ml	China
Flask with different size 25cm^2 , 75cm^2	
Gel loading tips	Bio Basic – Canada
Gloves	
Micropipettes various sizes(1000,100,10) µl	Extragene/Taiwan
Plastic scraper	China

3.3.2. Kits and Markers

Kits and markers are used in this study with their Manufacturer Company, and their origin are listed in Table (3-2).

Table (3.2): Kits and markers used in this study with their manufacturer Company and origin

Kits	Manufacturer Company/ Origin
G-Spin Total DNA Extraction Kit	Intron / Korea
Viral Nucleic Acid Extraction kit	Intron / Korea
Human KIR	Boster Biological Technology \USA
Human IL-1Ra	Boster Biological Technology \USA
Human IL 28	Boster Biological Technology \USA
Human TNF α	Boster Biological Technology \USA
Human IFN- γ	BT LAB \ CHINA
GoTaq® 1-Step RT-qPCR System	Promega \ USA

3.3.3. Reagents and Buffers

Reagents and buffers used in this study are listed in table (3.3).

Table (3.3): Reagents and buffers used in this study with their Manufacturer Company and origin.

Reagents	Manufactured Company (Origin)
Absolute Ethanol alcohol	Merck – Germany
Agarose E	Canada / Spain
DNA Ladder(100bp)	Intron /S. Korea
DNase	Zymo Research / USA
Proteinase K	Intron /S. Korea
Rad Safe Nucleic Acid staining solution	Zymo Research / USA
10X TBE DNA Sequencing Grade	Intron /S. Korea

3.3.4. Contents of Patho Gene-spinTM DNA/RNA Extraction Kit

The contents of the Patho Gene-spinTM DNA/RNA Extraction Kit are listed in Table (3.4).

Table (3.4). Contents of Patho Gene-spinTM DNA/RNA Extraction Kit

Reagents	Amount
Binding Buffer	(30 ml)
Elution Buffer (20 ml).	(20 ml)
Lysis Buffer	(35 ml)
Spin Columns inserted into collection tubes.	(2.0ml tubes) (50 columns)
Washing Buffer A	(30 ml)
Washing Buffer B2 was added 40 ml of EtOH before use.	(10 ml)

3.3.5. Product Components and Storage Conditions

The product components and storage conditions of GoTaq® 1-Step RT-qPCR Master Mix are listed in table (3.5).

Table (3.5): The product components and storage conditions

GoTaq® 1-Step RT-qPCR Master Mix	
All components stored between -30^{0}C to -10^{0}C Each system contains sufficient reagents for 500 × 20µl reactions. Includes:	
• GoTaq® qPCR Master Mix, 2X	5 × 1ml
• GoScript™ RT Mix for 1-Step RT-qPCR	225µl
• CXR Reference Dye, 30µM	200µl
• MgCl2, 25mM	750µl
• Nuclease-Free Water	2 × 13ml

3.3.6. Assembling the GoTaq® 1-Step RT-qPCR Reaction Mix.

Prepare the reaction mix (minus RNA template) by combining the GoTaq® qPCR Master Mix, GoScript™ RT Mix, PCR primers, and Nuclease-Free Water as described in Table (3-6).

Table (3.6): Assembling the GoTaq® 1-Step RT-qPCR Reaction Mix.

Component	**Volume**	**Final Concentration**
GoTaq® qPCR Master Mix, 2X	10µl	1X
GoScript™ RT Mix for 1-Step RT-qPCR (50X)	0.4µl	1X
Forward Primer (20X)	1µl	50–300nM
Reverse Primer (20X)	1µl	50–300nM
RNA template	2 µl	nM
CXR Reference Dye (optional)	0.33µl/20µl reaction	500nM
Nuclease-Free Water	to a final volume of 20µl	

3.3.7. Kit contents of G-Spin total DNA extraction

The contents of G-Spin total DNA extraction kit are listed in table (3.7).

Table 3.7: List of reagents and buffers of G-Spin total DNA extraction kit used in this study.

Label	Contents 50 Columns	Contents 200 Columns
Buffer CL	25 ml	90 ml
Buffer BL 1	25 ml	90 ml
Buffer WA1	40 ml	160 ml
Buffer WB 2	14 ml	56 ml
Buffer CE 3	20 ml	40 ml
Spin Column4 / Collection Tube 5	50 ea	200 ea
RNase A (Lyophilized powder) 6	3 mg x 1 vial	3 mg x 4 vials
Proteinase K (Lyophilized powder) 6	22 mg x 1 vial	22 g x 4 vials

3.3.8. Reagent provided of ELISA kits to evaluate TNF-α, IFN-γ, KIR, IL-1Ra and IL-28 levels.

Reagent provided of ELISA kits to evaluate TNF-α, IFN-γ, KIR, IL-1Ra, and IL-28 levels in the serum of patients with brain tumors as well as apparently healthy control groups are listed in table (3.8).

Table (3.8): List of reagents and buffers of IL-28, IL-1Ra, TNF-α, IFN-γ and KIR ELISA kits used in this study.

Components	Quantity (96 tests)
Standard Solution (96 ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated human TNF-α Antibody Biotinylated human IFN-γ Antibody Biotinylated human KIR Antibody Biotinylated human IL-1Ra Antibody Biotinylated human IL-28 Antibody	1ml x1 1ml x1 1ml x1 1ml x1 1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

3.3.9. Primes of HTLV-1, TNF-α, IFN-γ, KIR, IL-1Ra and IL-28 SNPs.

Primers set used in this study to detect the HTLV-1; SNPs of TNF-α, IFN-γ, KIR , IL-1Ra, and IL-28 polymorphism with their product size and source as well as origin are listed in Table (3-9).

Table 3.9: Primers sets that are used for detection of HTLV-1, IL-28, IL-1Ra, TNF-α, IFN-γ and KIR genes polymorphism.

Gene	Sequence (5′-3′)	Product size (bp)	Source/ origin	References
HTLV-1LTR-gag	OF- AACTAGCAGGAGTCTATAAAAGCG OR- AAAGATTTGGCCCATTGCCTAG	RT-PCR	IDT\ USA	van Tienen *et al.*,2012
HTLV-1LTR-gag	IF- ACAGTTCAGGAGGGGGCTC IR- TAGGGAATAAAGGGGCGCTC	RT-PCR	IDT\ USA	van Tienen *et al.*,2012
HTLV-1Tax LTR	OF- ACTCACACGGCCTCATACAG OR- ACGCAGTTCAGGAGGCAC	RT-PCR	IDT\ USA	van Tienen *et al.*,2012
HTLV-1Tax LTR	IF- CTGTTTGAAGAATACACCAACATCC IR- CTCAACCGGCGTGGATGG	RT-PCR	IDT\ USA	van Tienen *et al.*,2012
IL-28	F-TCCTCCAATCCCACCAGGAT R-GGTCTGTGAGCTCTGAGCAG	604 bp	IDT\ USA	Designed
IFN-γ	F-TGTTTCGAGGTCGAAGAGCAT R- GCCTGGTGCTTCCAAATATTGT	522 bp	IDT\ USA	Designed
TNF-α	F- GCTCATGGGTTTCTCCACCA R-AGGGGAAATGGAGACGCAAG	528 bp	IDT\ USA	Designed
KIR	F-GAGACAGACACCAGCAAGGG R-AGACTGACTTGCTGAGGTTTGT	530 bp	IDT\ USA	Designed
IL-1Ra	F- CCCAGCTCAGTTCTCTGCAT R- AAATGTCAAGCGCATGGAGC	477 bp	IDT\ USA	Designed

OF, outer forward; OR, outer reverse; IF, inner forward; IR, inner reverse

3.4. Methods

3.4.1. Detection of *Human-T-Lymphotropic Virus Type 1* by Real-Time Polymerase Chain Reaction (RT-PCR)

Real-time PCR (qPCR) is based on two major processes: **Firstly, isolation** of viral genomic RNA from specimens, and **Secondly**, Reverse transcriptase to convert viral RNA to cDNA and amplify for each sample. In real-time PCR (qPCR), the accumulating amplified product can be detected at each cycle with fluorescent dyes. This increasing signal allows to achievement of sensitive detection and quantification of pathogens.

3.4.1.1. Extraction of viral nucleic acid from clinical specimens

I. Extraction of viral nucleic acid from tissue samples.

A. Specimens preparation:

Brain tissue with 0.8 mm in diameter that was taken from various brain regions were frozen at -80°C until nucleotide extraction. After that, the tissue was cut into small pieces and gridded to ensure rapid lysis and high yields. Weight the appropriate tissue amount was weighted and placed in a 1.5 ml microfuge tube. Using more than the recommended amounts will not lead to better yields and/or purity. If using more than recommended is required, split the sample into 2 or more preps.

B. Assay Procedure:

The procedure was carried out in accordance with the manufacturer's instruction with some modifications as following:

1. Twenty –five mg (25 mg) from gridding brain tissue was transferred into 1.5ml micro centrifuge tube.
2. A 600-µl Lysis buffer was added, then the lysate was mixed by vortex for (25sec). Mixture was incubated at room temperature for (15 min). DNase (20 µl) was added in order to remove viral DNA genome (modified).
3. Six hundred µl from binding buffer was added and completely mixed well by gently vortex. This step is conducive efficient passage of cell lysates through a column and to increase binding onto column resins and important for effective deproteinization.
4. The lysates of cell was placed in a spin column that provided 2m collection tube.
5. Lysates was loaded on the column and centrifuged for 2 min at 13,000 rpm.
6. Solution in collection tube was discarded and placed the spin column back in the same (2ml) collection tube .
7. A 500 µl of washing buffer A was added to spin column and centrifuged for 2 min at 13,000 rpm.
8. The solution was discarded in a collection tube and placed the spin column back in the same (2ml) collection tube and centrifuged for 2 min at 13,000 rpm and then discarded solution.
9. Five hundred µl of washing buffer B was added to the spin column and centrifuged for 1min at 13,000 rpm.
10. The solution was discarded in the collection tube and placed the spin column back in the same (2ml) collection tube. After that it was centrifuged for 1min at 13,000 rpm. It is important to dry the membrane since residual ethanol may interfere with downstream reactions.
11. Spin column was place in an RNase-free (1.5ml) microcentrifuge tube; then 50 µl of Elution buffer was added directly onto the membrane and was incubated at RT for 2min, then centrifuged for 2min at 13,000 rpm.

12. At this stage, the supernatant was containing viral genome (RNA).

II. Extraction of Viral Nucleic Acid from Blood samples.

The procedure was carried out in accordance with the manufacturer's instruction with some modification as following:

1. Three hundred µl from blood plasma was transferred into 1.5ml micro centrifuge tube.
2. Six hundred µl Lysis buffer was added, then the lysate mixed by vortex for (25sec). Mixture was incubated at room temperature for (15 min).
3. Six hundred µl from binding buffer was added, and completely mix well by gently vortex. This step is conducive to efficient passage of cell lysates through a column and to increase binding onto column resins and it is important for effective deproteinization.
4. The lysates of cell were placed in a spin column that provided (2ml) collection tube.
5. Lysates was loaded on the column and centrifuged for 2 min at 13,000 rpm.
6. Solution was discarded in collection tube and place the spin column back in the same (2ml) collection tube.
7. Five hundred µl of washing buffer A was added to spin column and centrifuged for 2 min at 13,000 rpm.
8. The solution in collection tube was discarded and placed the spin column back in the same (2ml) collection tube and centrifuged for 2 min at 13,000 rpm and then discarded solution.
9. Five hundred µl of washing buffer B was added to the spin column and centrifuged for 1min at 13,000 rpm.

10. The solution in the collection tube was discarded and placed the spin column back in the same (2ml) collection tube. DNase (20 µl) was added in order to remove viral DNA genome (modified). Centrifuged for 1min at 13,000 rpm. It is important to dry the membrane since residual ethanol may interfere with downstream reactions.
11. Spin column was placed in an RNase-free (1.5ml) micro- centrifuge tube; then 50 µl of Elution buffer was added directly onto the membrane and was incubated at RT for 2min, then centrifuged for 2min at 13,000 rpm.
12. At this stage, the supernatant contained viral genome (RNA), then stored at -20°C.

3.4.2. GoTaq® 1-Step RT-qPCR Protocol

1. **Materials to Be Supplied by the User**

- Real-time PCR instrument and related equipment (i.e., optical-grade PCR plates and appropriate plate covers)
- Sterile, aerosol-resistant pipette tips.
- Nuclease-free pipettors dedicated to pre-amplification work.
- RNA template.
- qPCR primers.

2. **Adding CXR Reference Dye to the GoTaq® qPCR Master Mix (Optional)**

Some real-time PCR instruments require higher levels of CXR Reference Dye; For high reference dye instruments, add CXR Reference Dye to achieve a high dye concentration (500nM), as follows:

A. The GoTaq® qPCR Master Mix was thawed. Do not thaw the master mix at temperatures above room temperature.
B. The GoTaq® qPCR Master Mix was vortexed for 3–5 seconds to mix.
C. The instrument designated as a high reference dye instrument, then added 0.33µl per 20µl reaction for a final concentration of 500nM.
D. Vortexed for 3–5 seconds to mix.

3. Assembling the GoTaq® 1-Step RT-qPCR Reaction Mix

The final reaction volume in this protocol is 20µl. The volumes given here may be scaled for larger or smaller reaction volumes.

A. The GoTaq® qPCR Master Mix and Nuclease-Free Water were thawed. Not that the GoTaq® qPCR Master Mix at elevated temperatures (i.e., above room temperature).
B. The GoTaq® qPCR Master Mix was vortexed for 3–5 seconds to mix. The vortex at low speed to avoid aeration.
C. The number of reactions were added to be set up, included negative control reactions. Added 1 or 2 reactions to this number to compensate for pipetting errors. While this approach was required using a small amount of extra reagent, it ensures that you will have enough reaction mixed for all samples.

4. Assembling the GoTaq® 1-Step RT-qPCR Reaction Mix (continued)

Prepared the reaction mix (minus RNA template) by combining the GoTaq® qPCR Master Mix, GoScript™ RT Mix, PCR primers, and Nuclease-Free Water as described in table (3.6). The RNA template was added. Vortexed briefly to mix.

Note: The primer concentrations should be optimized for each primer combination.

A. The appropriate volume of the reaction mix was added to each PCR tube

or well of an optical-grade PCR plate.

B. The RNA template (or water for the no-template control reactions) was added to the appropriate wells of the reaction plate.

C. The tubes or optical plate was sealed and centrifuged briefly to collect the contents of the wells at the bottom. Protected from extended light exposure or elevated temperatures. The samples are ready for thermal cycling.

3.4.2.1. Thermal Cycling

The cycling parameters below are offered as a guideline and may be modified as necessary for optimal results as shown in Table (3.10).

Table (3.10): Standard-Cycling Conditions

Step	Cycles	Temperature	Time
Reverse transcription	1	≥37°C	15 minutes
Reverse transcriptase inactivation and GoTaq® DNA Polymerase activation	1	95°C	10 minutes
Denaturation		95°C	10 seconds
Annealing and data collection	40	60°C	30 seconds
Extension		72°C	30 seconds

Using the instrument optical settings established for SYBR® Green I assays with GoTaq® qPCR Master Mix.

3.5. Detection of IL-28, TNF-α, IFN-γ, IL1Ra and KIR genes by Sequencing

Total DNA for SNPs of IL-28, TNF-α, IFN-γ, IL1Ra and KIR genes polymorphism were extracted from the tissue of patients using sequencing technique.

3.5.1. Principles of Assay

Is based on two major processes: isolation of total DNA from specimens and Polymerase chain reaction (PCR) it allows the amplification of a target region from a DNA template by using specific oligonucleotides. Finally, the PCR products of IL-28, TNF-α, IFN-γ, IL1Ra and KIR genes to detect SNPs were sent to Macrogene \ Korea to detect the variation of IL-28, TNF-α, IFN-γ, IL1Ra and KIR SNPs.

3.5.2. Extraction of Total DNA from Clinical Samples

3.5.2.1. Assay Procedure

The procedure is carried out in accordance with the manufacturer's instruction as following:

I .Extraction of total DNA from Tissues Samples

1. Twenty-five mg (300 µl) of grinded tissue sample was measured, and then was transferred into 1.5 ml tube using a spatula.
2. Two hundred µl Buffer CL, 20 µl Proteinase K and 5 µl RNase A solution were added into sample tube and mixed by vortex vigorously.
3. The lysate was incubated at 56 C^0 for 30 min.
4. Two hundred µl of Buffer BL was added into upper sample tube and mixed thoroughly. Then incubated the mixture at 70C^0 for 5min. This step is

important in order to ensure to assure efficient lysis, it is important that the lysate sample and Buffer BL are mixed thoroughly.

5. The sample tube was centrifuged at 13,000 rpm for 5 min to remove un-lysed tissue particles. Then carefully was transferred 350 ~ 400 µl of the supernatant into a new 1.5 ml tube
6. The 1.5 ml tube was centrifuged briefly to remove drops from the inside of the lid.
7. Two hundred µl of absolute ethanol was added into the lysate and then mixed well by pulse vortex. After then, the mixture was centrifuged to remove drops from inside of the lid.

   This step is an equilibration step for binding genomic DNA to the column membrane. In addition, this step conduces to pass cell lysate efficiently through a column.
8. The mixture from step 7 was applied carefully to the spin column (in a 2 ml collection tube) without wetting the rim, closed the cap, and centrifuged at 13,000 rpm for 2 min. Discarded the filtrate and placed the spin column in a new (2 ml) collection tube (additionally supplied).
9. Seven hundred µl of Buffer WA was added to the spin column without wetting the rim and centrifuged for 1 min at 13,000 rpm. discarded the flow-through and reused the collection tube.
10. Seven hundred µl of Buffer WB was added to the spin column without wetting the rim and centrifuged for 1 min at 13,000 rpm. Discarded the flow-through and placed the column into a new (2.0 ml) collection tube (additionally supplied). Then it was centrifuged again for an additional 1 min to dry the membrane. Discarded the flow-through and collection tube altogether.

11. The spin column was placed into a new (1.5 ml) tube and added 50 µl of Buffer CE directly onto the membrane. Then it was incubated for (1 min) at room temperature and then centrifuged for 2 min at 13,000 rpm to elute.

II. Extraction of total DNA from Blood Samples

1. Two hundred µl of whole blood was pipetted into a (1.5 ml) micro-centrifuge tube.
2. Fourty-µl proteinase K solution and 5 µl of RNase A solution were added into the specimen tube and gently mixed.
3. Two hundred µl of Buffer BL was added into the upper specimen tube and mixed thoroughly. This step is important in order to ensure effective decomposition, and it is important that the BL sample and buffer blood are thoroughly mixed to give a dissolution solution.
4. The mixture was placed at room temperature for 2 minutes.
5. The lysate was incubated at 56°C for 10 min. For complete lysis, mix 3 or4 times during incubation by inverting tube. If it breaks down perfectly, the red color of lysate became dark green.
6. The 1.5 ml tube was centrifuged briefly to remove drops from inside the lid.
7. Two hundred µl of absolute ethanol was added into the lysate, and then mixed well by pulse vortex. After mixing, briefly centrifuged the (1.5 ml) tube to remove drops from inside of the lid. This step is an equilibration step for binding genomic DNA to column membrane. In addition, this step conduces to pass efficiently cell lysate through a column.
8. The mixture from step 7 was applied carefully to the spin column (in a 2 ml collection tube) without wetting the rim, close the cap, and centrifuged at 13,000 rpm for 1 min. Discarded the filtrate and placed the spin column in a new (2 ml) collection tube (additionally supplied).

9. Seven hundred (700) microliters of Buffer WA was added to the spin column without wetting the rim, and centrifuged for 1 min at 13,000 rpm. Discarded the flow-through and reused the collection tube.
10. Seven hundred µl of Buffer WB was added to the spin column without wetting the rim, and centrifuged for 1 min at 13,000 rpm. Discarded the flow-through and placed the column into a new (2ml) collection tube (additionally supplied), then it was centrifuged again for an additional 1 min to dry the membrane and discarded the flow-through and collection tube altogether.
11. The spin column was placed into a new (1.5 ml) tube (not supplied), and added (50 µl) of Buffer CE directly onto the membrane, and then incubated for (1 min) at room temperature and then centrifuged for 1 min at 13,000 rpm to elute, then stored at -20°C.

3.5.3. Estimation of the Extracted RNA and DNA Concentration and Purity

After extraction of viral RNA and DNA from samples; the concentration of RNA and DNA yield and purity are measured by using Mlite bio drop (England), by applying 5 µl of the extracted RNA and DNA in the instrument curette. Extracted with purity in between (1.7-1.9) at absorption wavelength 260/280 was included in this study, otherwise; RNA/DNA extraction of the sample was repeated.

3.6. Detection of IL-28, TNF-α, IFN-γ, IL1Ra, and KIR Genes Polymorphism by Polymerase Chain Reaction (PCR)

3.6.1 Primers Pairs Dilution

The primers source were from Bioneer primers are commonly transported in a lyophilized state. The units of a lyophilized primer are given as a mass in Pico moles. To create a stock of primers, one would reconstitute the primer in sterile,

nuclease-free water. The company supplies the amount of sterile, nuclease-free water to be added to each primer to obtain master stock (100Pmol/ml) that will be used again to obtain working stock. As following: The tube was spin down before opening the cap, and then the desired amount of water was added according to the oligos manufacturer to obtain 100 pmol/µl (Master Stock). Vortex properly to re-suspend the primers evenly. Then 10µl of the master stock was transfered to a 0.2ml Eppendorf tube that contains 90µl of sterile, nuclease-free water (Working Stock). The master stock is stored at -20 C° and the working stock is stored at -20 C°. Finally, the working stock was thawed on ice and vortex before using in PCR and then stored at -20 C°.

3.6.2. PCR Experiments

PCR amplification was done using a conventional thermal cycler (Biometra-Germany) as follows: Template DNA (about 2 µl) was added into PCR master mix tubes. Forward and reverse primers were added 1.5 µl into PCR master mix tubes (for each one). Distilled water was added to PCR Premix tubes to a total volume of 25 µl as the Table (3-11).

Table 3.11: Recommended volumes and concentration for applying PCR into AccuPower® PCR tubes.

No.	Content of PCR Reaction Mixture	Volume/ µl
1	Master mix	12.6 µl
2	Forward primers (each one of snps)	1.5 µl
3	Reverse primers (each one of snps)	1.5 µl
4	Template DNA	2 µl
5	Nuclease free water	7.4 µl
Total		**25 µl**

3.6.3. Thermal Cycles Condition

Reactions were placed in a thermal cycler (Biometra-Germany) that had been preheated to 94°C and beforehand set up to the desired cyclic conditions. The target regions of IL-28, TNF-α, IFN-γ, IL1Ra and KIR polymorphism were amplified using specific primers according to the mentioned conditions in Table (3-12).

Table 3.12: Amplification Conditions of -28, TNF-α, IFN-γ, IL1Ra and KIR Genes in Patients with Brain tumor.

Gene	Initial denaturation	Denaturation	Annealing	Extension C^0/	No. of cycles	Final extension
IL-28	95C^0 /5 min	95C^0 / 1 sec	58 C^0/45 sec	72C^0/1 min	40	72C^0/10min
IFN-γ	95C^0 /5 min	95C^0 / 1 min	60 C^0 /45sec	72C^0/2 min	40	72 C^0 /5min
TNF-α	95C^0 /5 min	95C^0 / 1 sec	57 C^0 /45sec	72C^0/1 min	40	72 C^0/5 min
KIR	95C^0 /5 min	95C^0 / 1 min	56.5C^0/45sec	72C^0/ 2min	40	72 C^0 /5min
IL1Ra	95C^0 /5 min	95C^0 / 1 sec	58 C^0 /45sec	72C^0/1 min	40	72 C^0 /5min

PCR products of target regions IL-28, TNF-α, IFN-γ, IL1Ra and KIR polymorphism were electrophoresed on 1.5% agarose at 75 V for 1h and visualized by safe stain. Photos were taken using a gel documentation system.

3.7. Agarose Gel Electrophoresis Technique

The agarose gel electrophoresis was performed according to the method of Robinson and Lafleche (2000). This technique was used to detect viral genomic, genomic DNA extracts, and PCR products.

3. 7.1 Preparation of Solutions and Buffers

I. Loading Buffer

The buffer was prepared from 0.25 % Bromophenol blue and 40% sucrose and stored at 4° C (Sambrook and Rushell, 2006).

II. TBE Buffer (1X)

To prepare 500 ml of 1X TBE buffer, 50 ml of TBE (10X) stock solution was mixed with 450 ml of dH_2O. The pH value was adjusted to 8 with concentrated HCl or 0.5 M tris base solution. Then the volume was completed to 500ml with dH_2O.

3.7.2 Gel Electrophoresis Protocol

1. Device setup: The casting gates were sited on the ends of the gel tray and locked in place firmly against the casting tray. This was done by engaging the "claws" of the gate in the recess of the sidewall of the tray. The comb was sited into the slots of the gel tray (1.0 mm above the base of the gel-casting tray) so that the sample wells were near the cathode.
2. Gel dissolving: 1g of agarose was dissolved in 100 ml of 0.5 X TBE solution by melting to 100°C to prepare 1% agarose gel for migrated genomic DNA extracts. At the same time, 1% or/and 2% agarose gel was prepared in 0.5 X TBE buffer for migrated PCR products.
3. Gel casting: After the agarose gel dissolved completely, let it cool to approximately 60°C and 2-3 µl of the safe stain stock solution was added; then, slowly pour the agarose into the gel-casting tray, and any air bubbles were

removed. The comb was positioned at approximately 1.5 cm from one edge of the gel. The agarose was allowed to solidify at room temperature for at least 30 min. After that, the claws were disengaged from the gel tray, and the comb was separated gently. Then the gel was placed in the gel tank in such a way that the wells should be on end with the cathode. 1X TBE buffer (depending on the purpose) was added to the buffer tank until it was about 5 mm above the top of the gel.

4. Loading the samples: Each 5µl of the genomic DNA sample was mixed with 3µl loading dye briefly and loaded into the wells. Whereas the PCR products were loaded without loading dye because the PCR master mix contained loading dye.
5. Gel electrophoresis conditions: After sample loading, the electric field was turned on at 5 V/cm (75V) for 60-120 min until the bromophenol blue dye reached the end edge of the gel.
6. The gel was photographed using a gel documentation system (Cleaver Scientific - UK).

3.8. Sequencing of Studied Markers

I. DNA Sequencing of PCR amplicons

The resolved PCR amplicons were commercially sequenced from forward termini according to the instruction manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI sequence files were further analyzed, ensuring that the annotation and variations were not because of PCR or sequencing artifacts. The virtual positions and other details of the retrieved PCR fragments were identified by comparing the observed DNA sequences of the investigated samples with the retrieved neighboring DNA sequences of the NCBI Blastn engine.

II. Interpretation of sequencing data

The sequencing results of the PCR products of different samples were edited, aligned, and analyzed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.2 (DNASTAR, Madison, WI, USA). The observed variations in each sequenced sample were numbered in PCR amplicons as well as in their corresponding position within the referring genome. The targeted SNP was visualized in the dbSNP database to check its details positioned according to its place in the reference genome.

III. Checking the details of SNPs

The determination of the previous deposition of the observed SNP was performed by viewing its corresponding dbSNP position. Then, the dbSNP position for the detected SNP was documented. The observed SNP was submitted to the dbSNP database to check its details. The targeted SNP position was checked in its corresponding reference genome to assess its frequency and effect in the dbSNP server.

3.9. Evaluation of the level of IL-28, TNF-α, IFN-γ, IL1Ra, and KIR in blood serum of patients and AHC groups.

The concentration of IL-28, TNF-α, IFN-γ, IL1Ra, and KIR in the serum of patients with brain tumors and healthy control groups were evaluated by enzyme-linked immunosorbent assay (ELISA).

I. Assay Procedure

1. All reagents were prepared, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay was performed at room

temperature.

2. The number of strips required for the assay was determined. The strips were inserted in the frames for use. The unused strips should be stored at 2-8°C.
3. Fifty-µl standard was added to the standard well. **Note**: antibody do not add to the standard well because the standard solution contains biotinylated antibodies.
4. Forty µl of the sample was added to sample wells and then added 10µl anti-TNF-α, IFN-γ, KIR, IL-1Ra, and IL-28 antibodies to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. Covered the plate with a sealer. Incubated at 60 minutes at 37°C.
5. The sealer was removed and washed the plate for 5 times with wash buffer. soaked wells with at least 0.35 ml wash buffer for 30 seconds for each wash. For automated washing, aspirated or decanted each well and washed 5 times with wash buffer. Blotted the plate onto paper towels or other absorbent material.
6. Fifty µl substrate solution A was added to each well and then 50µl substrate solution B was added to each well. plate covered and incubated with a new sealer for 10 minutes at 37°C in the dark.
7. Fifty µl from the stop solution was added to each well, and the blue color was changed into yellow immediately.
8. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

II. Calculation of The Results

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best-fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best-fit line can be determined by regression analysis.

III. Typical Data

This standard curve is only for demonstration purposes. A standard curve should be generated with each assay.

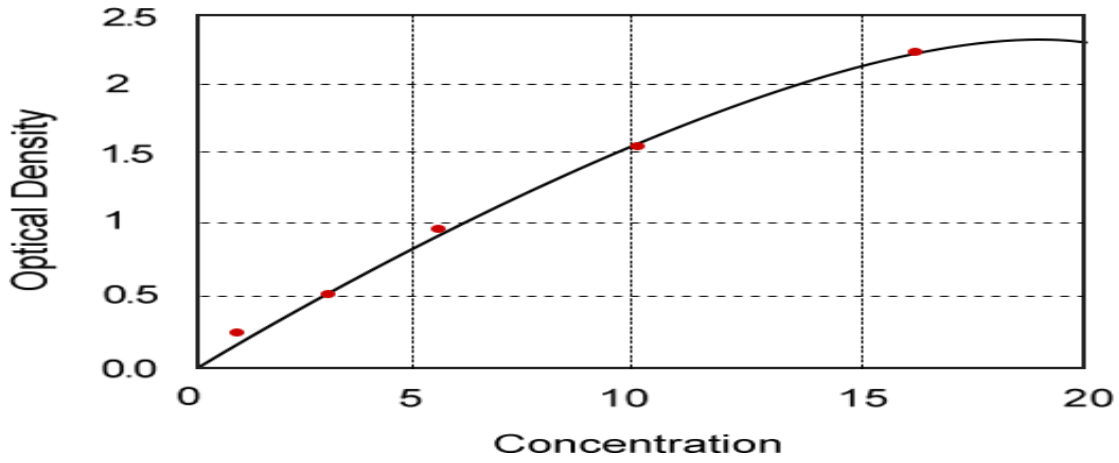

Figure (3.2): The standard curve for estimation the concentration of study parameter in sera of patients and control group

3.10. Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 3.06. Standard deviations are plotted as error bars for the data points on all figures two way ANOVA, One-way ANOVA, and Chi-square ($X^{2)}$ were done to establish

relationships of expression immunological variables levels according to the ELISA test results between patients with brain tumors and AHC.

The correlation matrix between the selected variables and *HTLV-I* infection in the current study was estimated by using Spearman's correlation coefficient analysis. Correlation coefficients were considered significant at P-values less than 0.05 by using GraphPad Prism version 3.06. Asterisk (*****) indicates that the differences were statistically significant compared to the control group with patient groups. The chi-square test (X^2) was used to compare the selected groups * $p<0.01$; *** $p<0.001$.

Hierarchical cluster analysis based on similarity coefficient was used in this study to identify the relatively homogenous results using expression immunological variables levels according to the ELISA test results between patients with brain tumors and AHC. Also used PRIMER-E7 software package (http://www.primer-e-.com) for primer design (Clarke *et al.,* 2014).

The Hardy-Weinberg equilibrium (HWE) was used to examine the allele and genotype frequency distributions. HWE is a crucial quality assurance step in population-based genetic association studies, which have been shown to be effective in identifying genes linked to a wide range of complex human disorders with significant public health implications (Namipashaki *et al*., 2015).

The Bio Edit software was used for sequence alignment and Swiss Model and Pymol 2_5_2 Windows 64 program were used to determine the protein folding of human genes in the present study.

Chapter Four

Results

4. The Results

4.1. Distribution of Patients with Brain Tumor and Control Groups According to Their Age

The tissue samples were related to patients with brain tumors whose ages ranged from 2 years to 85 years, with a mean age of 50.7 ± 10.4 years, as compared to 47.9 ± 12.6 years for their control counterparts. However, between these age groups, no significant variations were detected (P > 0.05), as shown in table (4.1).

Table (4.1): Distribution of patients and control group according to their age.

Study Group	No.	Mean Age (Years)	S.D	S.E	Min	Max
Brain tumors	75	50.7	10.4	2.0	2	85
Control	75	47.9	12.6	2.9	16	71
Statistical Analysis	**Non-significant (P > 0.05) = 0.06**					

4.2. Sex distribution in study groups

Males accounted for 41 (54.7 %) of brain tumor cases, while females accounted for 34 (45.3 %). The ratio of males to females was 1.2:1, while the control group had a sex distribution of 45 (60%) males and 30 (40%) females. The brain tumors and control group showed a significant difference (P<0.05) in the statistical analysis according to their sex distribution as shown in table (4.2).

Table (4. 2:) Distribution of study groups according to their sex

Sex	Brain Tumors		Control		P-value
	No.	%	No.	%	
Male	41	54.7	45	60	0.001*
Female	34	45.3	30	40	0.002*
Total	75	100	75	100	

***Statistical significant**

4.3. Distribution of study brain tumors group according to their age stratum and sex

Regarding the age of patients with brain tumors, 6.7 % of cases are between the ages of 2 and 18 (3 male and 2 female), 14.7 % are between the ages of 19 and 35 (6 male and 5 female), 22.6 % are between the ages of 36 and 52 (9 male and 8 female), 28% are between the ages of 53 and 69, (12 male and 9 female), and 28 % are between the ages of 70 and 85 (11 male and 10 female). The highest male frequency (12) was found in the 53–69 year age group, whereas the highest female frequency (10) was found in the 70–85 year age group. The brain tumors and control group showed a significant difference ($P<0.05$) in the statistical analysis according to their age stratum and sex as shown in table (4.3).

Table (4.3): Patients with brain tumors according to their age and sex.

Age	Sex		Total		P-value
	Male	Female			
	No.	No.	No.	%	
2-18	3	2	5	6.7	0.06
19-35	6	5	11	14.7	0.06
36-52	9	8	17	22.6	0.06
53-69	12	9	21	28	0.04*
70-85	11	10	21	28	0.06
Total	41	34	75	100	0.03*

***Statistical significant**

4.4. The Grading of the studied brain tumors

In this study, grade I was found in 37 cases (49.3%) of brain tumor cases (20 males and 17 females), while grade II was found in 17 cases (22.7%) (9 males and 8 females), grade IV were seen in 13 cases which were (17.3%) (7 males and 6 females); finally, grade III were seen in 8 cases only (10.7%) of brain tumor group (5 males and 3 females) as in Table (4.4). There were statistically significant differences ($P \leq 0.05$) between groups of brain tumors based on their grading.

Table (4.4): Distribution of brain tumors according to grading

Grading of brain tumors	Sex		Total		P-value
	Male	Female			
	No.	No.	No.	%	
I	20	17	37	49.3	0.04*
II	9	8	17	22.7	0.06
III	5	3	8	10.7	0.05*
IV	7	6	13	17.3	0.05*
Total brain tumors	41	34	75	100	0.03*

*Statistical significant

4.5. Rating of brain tumors according to their types

Figure (4.1) shows that 21 cases (28%) of brain tumors as Pilocytic Astrocytoma (11 males and 10 females), 15 cases (20%) as Meningotheliomatous Meningioma (8 males and 7 females), 13 cases (17.3%) as Glioblastoma Multiforme and Diffuse Fibrillary Astrocytoma (7 males and 6 females) for each, 6 cases (8%) as Anaplastic Oligodendroglioma (3 males and 3 females), 4 cases (5.4 %) as A typical Meningioma (3 males and 1 female), and 3 cases (4 %) as Transitional Meningioma (2 males and 1 female). There were statistically significant differences ($P \leq 0.05$) between groups of brain tumors based on their types.

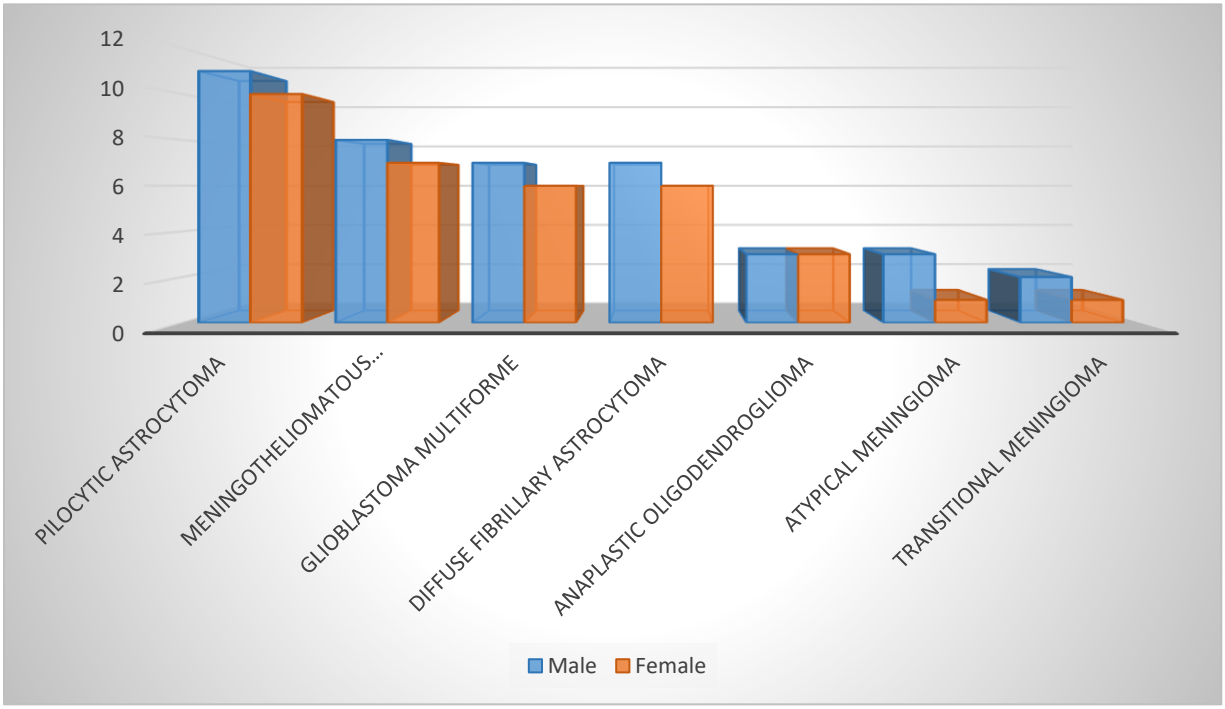

Figure (4.1): Rating of brain tumors according to their typing.

4.6. Detection of *Human T Lymphotropic Virus-I (HTLV-1*) by Real-Time Polymerase Chain Reaction Technique (rt.PCR)

4.6.1. Extraction of viral nucleic acid

Out of 75 brain tumor specimens involved in this study, 50 (66.7%) were found to have a viral infection. Twenty-five brain tumor specimens without a viral genome were detected in this study, accounting for 33.3% of the total number of patients Figure (4.2). In the control group, only 5 (3 from blood as well as 2 from brain tissues) out of 75 specimens have viral nucleic acid Table (4.5). The statistical analysis of these results revealed significant differences ($p = 0.01$).

Table (4.5): Viral genome extraction in brain tumors patients and control groups.

Viral Genome		Study Groups		P-value
		Brain Tumors No. (75)	Control No. (75)	
Positive	No.	50	5	P=0.01* Hig. Sign. (P<0.05)
	%	66.7 %	6.6%	
Negative	No.	25	70	
	%	33.3%	93.4%	
Total	No.	75	75	
	%	100%		

*Statistical significant

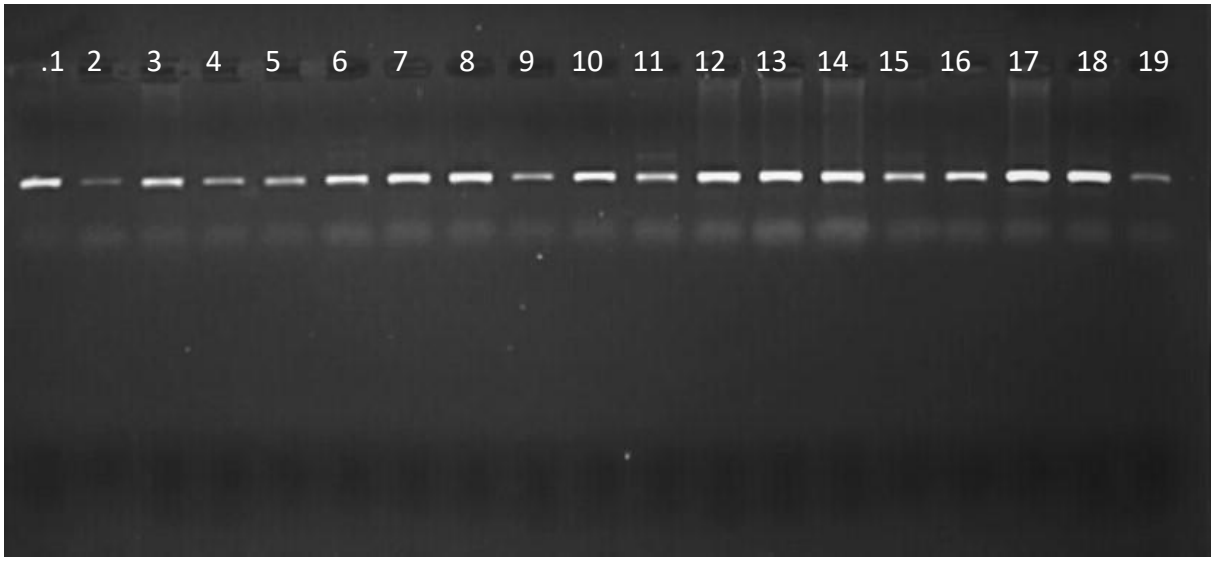

Figure (4.2). Agarose gel electrophoresis of Viral Genome from Patients with Brain Tumor,1 % Agarose Gel, TBE 1X, at power 75 Volt for 45 min, Lanes (1-19) were Positive.

4.6.2. HTLV-1 Genome Detection Using Qualitative qRT.PCR:

The positive results according to qRT-PCR shows 42 % (21 out of 50 cases) as positive while 58% (29 out of 50 cases) as negative, as shown in table (4.6) as well as figures (4-3A and B). While, none positive signals for HTLV-1 in control group. There were significant differences (p = 0.04) among patients and control groups.

Table (4.6): Positive signals of HTLV-1 using qRT-PCR in samples of patients with brain tumors and control groups.

HTLV-1 Genome	Positive No. (%)	Negative No. (%)
Patients with Brain tumors no.(50 cases)	21 (42%)	29 (58%)
Control groups No.(5 cases)	0 (0.0%)	5 (100%)
P-value	*P=0.04* Sign. (P<0.05)*	

***Statistical significant**

Figure (4.3 A and B) both of them show line positive control and line negative control and also positive samples and negative samples; according to the detection kit (qRT-PCR) of HTLV-I used in this study, the curves that represent positive samples appear between the line positive control and line negative control.

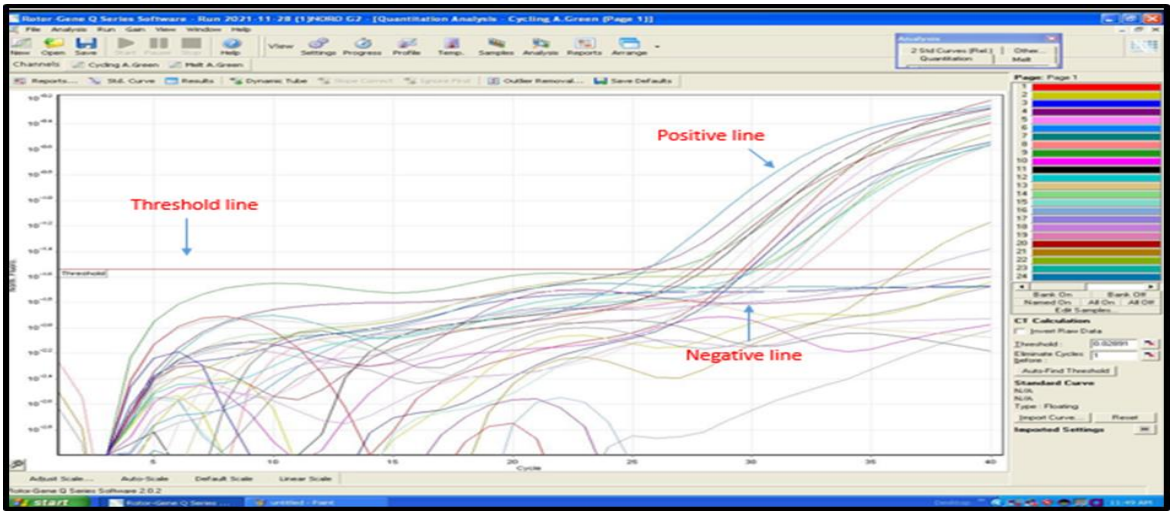

Figure (4.3A): The qRT-PCR detection results of HTLV-1 in brain tumor tissues.

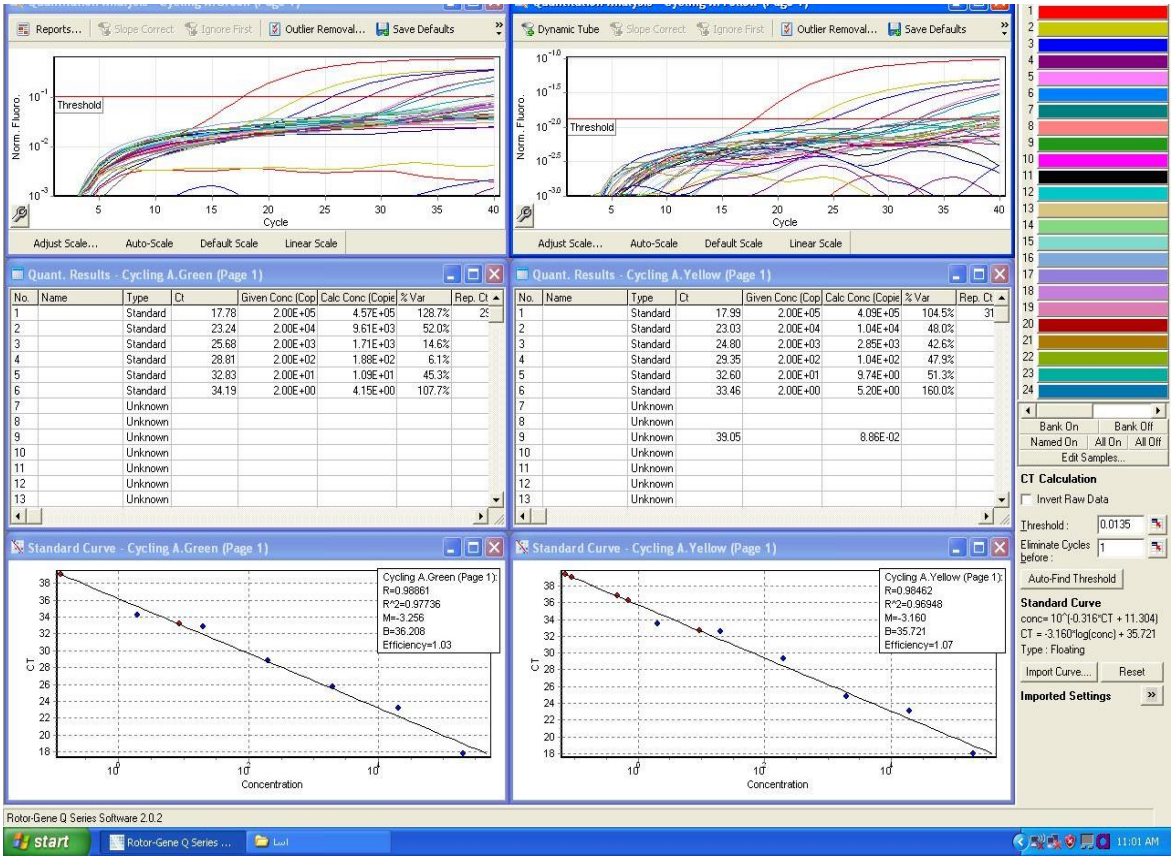

Figure (4.3.B): Qualitative and standard curve for detection of *HTLV-1* by RT-PCR.

4.6.2.1. The HTLV-1 Results in Patients with Brain Tumors by Age Stratum.

The most infected brain tumor tissues with RNA-HTLV-1 are related to the age stratum (70-85 years), which accounted for 33.3 % (7 out of 21 tissues), while the age stratum (53-69 years), (36-52 years), (19-35 years) and (2-18 years) each accounted for 23.8 % (5 out of 21 tissues); 19.0 % (4 out of 21 tissues); 14.3 % (3 out of 21 tissues); and 9.5 % (2 out of 21 tissues), respectively. Significant differences ($P<0.05$) were found when these age groups were compared statistically as shown in Table (4.7).

Table (4.7). RT-PCR signal frequency of *HTLV-1* among brain tumor tissues according to age groups.

	Years	*HTLV-1*			P value
		No.	Positive	Negative	
Age Group	2-18	5	2	3	0.03*
		6.7%	9.5%	5.5%	
	19-35	11	3	8	0.04*
		14.7%	14.3%	14.8%	
	36-52	17	4	13	0.03*
		22.6%	19.0%	24.1%	
	53-69	21	5	16	0.03*
		28%	23.8%	29.6%	
	70-85	21	7	14	0.03*
		28%	33.3%	25.0%	
Total		75	21	54	0.02**
		%100	28%	72%	

***Statistical significant**

**** Highly statistically significant**

4.6.2.2. The HTLV-1 Results in Patients with Viral RNA by Sex.

Table (4.8) shows the percentage of brain tumor tissues that have positive HTLV-1-RT-PCR results based on the sex of patients, with males accounting for 61.9% (13 out of 21 cases) and females accounting for 38.1% (8 out of 21 cases). In the brain tumors group, statistical analysis revealed significant differences in sex in relation to positive HTLV-1 qRT-PCR (P<0.05).

Table (4.8). *HTLV-1*- infection rates in brain tumor patients based on their sex

Brain tumor patients	HTLV-1- Infection	
	No.	%
Male	13	61.9%
Female	8	38.1%
Total	21	100%
The analysis Statistical	(P<0.05)= 0.03*	

*Statistical significant

4.6.2.3. Distribution of HTLV-1 Infection according to the types of brain tumors

Table (4.9) shows positive *HTLV-1* qRT-PCR detection results from patients with various forms of brain tumors, which were 10%, 6%, 18%, 4%, and 4% of Pilocytic Astrocytoma;q Meningotheliomatous Meningioma; Glioblastoma Multiforme; Diffuse Fibrillary Astrocytoma and Transitional Meningioma, respectively, showed positive qRT-PCR results for *HTLV-1* detection. The

statistical analysis of different types of brain tumors with *HTLV-1* positive showed significant differences ($p<0.05$).

Table (4.9). Frequency of brain tumor types with HTLV-1- positive qRT-PCR results

Type of tumor	Brain Tumor		HTLV-1 Positive		P-value
	With viral genome	Without viral genome			
	No.	No.	No.	%	
Pilocytic Astrocytoma	13	8	5	10%	0.04*
Meningotheliomatous Meningioma	11	4	3	6%	0.04*
Glioblastoma Multiforme	12	1	9	18%	0.04*
Diffuse Fibrillary Astrocytoma	7	6	2	4%	0.06
Anaplastic Oligodendroglioma	3	3	0	0.00	0.07
Atypical Meningioma	1	3	0	0.00	0.05*
Transitional Meningioma	3	0	2	4%	0.04*
Total	50	25	21	28%	0.03*

***Statistical significant**

4.7. Molecular study

4.7.1. Detection of *TNF-ALPHA* gene polymorphisms

In the present study, the *TNF-ALPHA* genetic sequences in chromosome 6. Concerning the currently investigated 528 bp amplicons of this gene, the NCBI BLASTn engine showed about 99.5% sequence similarities between the sequenced samples and the intended reference target sequences, which cover entirely the exon-1 sequences for the tumor necrosis factor according to the previous annotations of the human genome (GenBank acc. MH180383.1). By comparing the observed DNA sequences of these investigated samples with the retrieved DNA sequences (GenBank acc. MH180383.1). With regard to G>A, it was demonstrated that this SNP was identified in a heterozygous (G/A) pattern as well as a homozygous (G/G) pattern.

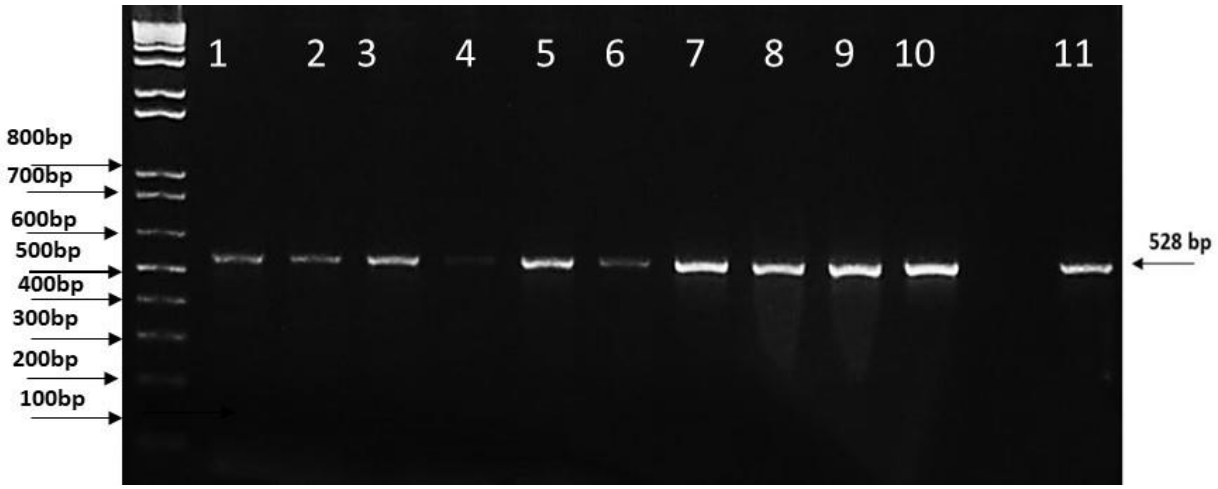

Figure (4.4): PCR amplification with specific primers for *TNF-α* gene, with conditions, 1.5% agarose, 75 V, for 1h, 5 µl in each well stained with a red safe solution, Lanes (1-11) were Positive.

The alignment results of the 528 bp samples revealed the presence of only one variation in all of the analyzed samples compared with the referring reference DNA sequences.

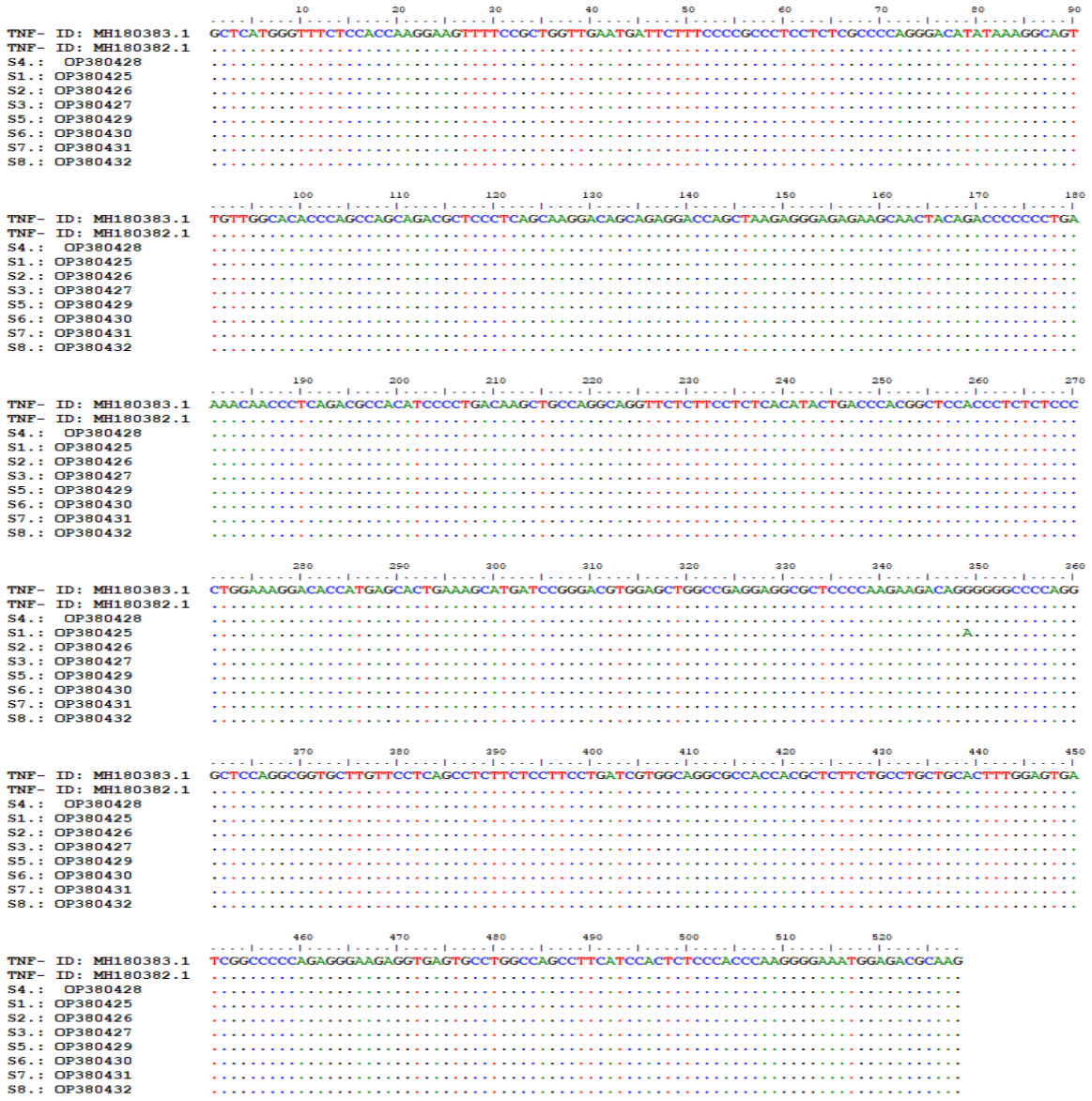

Figure (4.5): The Alignment Results of the *TNF-ALPHA* Gene according to BioEdit 7.2 software.

4.7.1.1. Genotyping of TNF- alpha (TNF-α) Polymorphism

To summarize the results obtained from the sequenced 528 bp fragments, the detailed positions of the observed variations are described in the NCBI reference sequences, as shown in Table (4.10). However, the substitution of G with A was not mentioned in the deposited SNP and this SNP was not reported in any publication yet.

The results showed that DNA polymorphism distribution was according to GG; GA and AA, which were 40%, 30%, and 30%, respectively, in patients with brain tumors and 80%; 4% and 16%, respectively, in the control group. The difference in frequency of genotype distribution of the polymorphism between patients and control groups was statistically significant Table (4.10).

Table (4.10). Genotyping of *TNF-ALPHA* gene (528 bp) in comparison with the NCBI referring sequences (GenBank acc. no. NC_000006.12).

TNF-ALPHA	Brain tumors No. (%)	Control No. (%)	Chi-square	p-value	Odd ratio (CI95)
GG	20 40%	20 80%	Reference		
GA	15 30%	1 4%	9.33	0.002*	1.06 (0.58-2.55)
AA	15 30%	4 16%	4.47	0.034*	1.26 (0.07-2.94)
Allele					
G	55	41			
A	45	9	10.54	0.001*	1.26 (0.51-2.61)

***Statistical significant**

After that, the sample sequences were submitted in NCBI, and the accession number of nucleotide sequences of TNF-α as new recording as shown in appendix (1): BankIt2620154; OP380425; OP380426; OP380427; OP380428; OP380429; OP380430; OP380431; OP380432.

4.7.1.2. Protein folding of *TNF-alpha* gene

When translating the DNA sequence using by Bio Edit program version 7.2.5 according to the reference sequence alignment of the human TNF-alpha gene ID: NP_000585.2. The results appeared to change the amino acid residues of the TNF-alpha protein as shown in figure (4.6). The amino acid substitution was the substitution of glycine to glutamic acid in position 22, which may affect or modified the tertiary structure of the protein; subsequently, the function may be affected figures (4.7).

Figure (4.6). Protein sequences alignment of the genotyped sample with their corresponding reference sequences of the 233 amino acids of the *TNF-alpha* gene. The symbol "MH180383.1" refers to the NCBI referring sequence. The "S" refers to the genotyped sample. This SNP caused an amino acid substitution of glucin to glutamine in position 22.

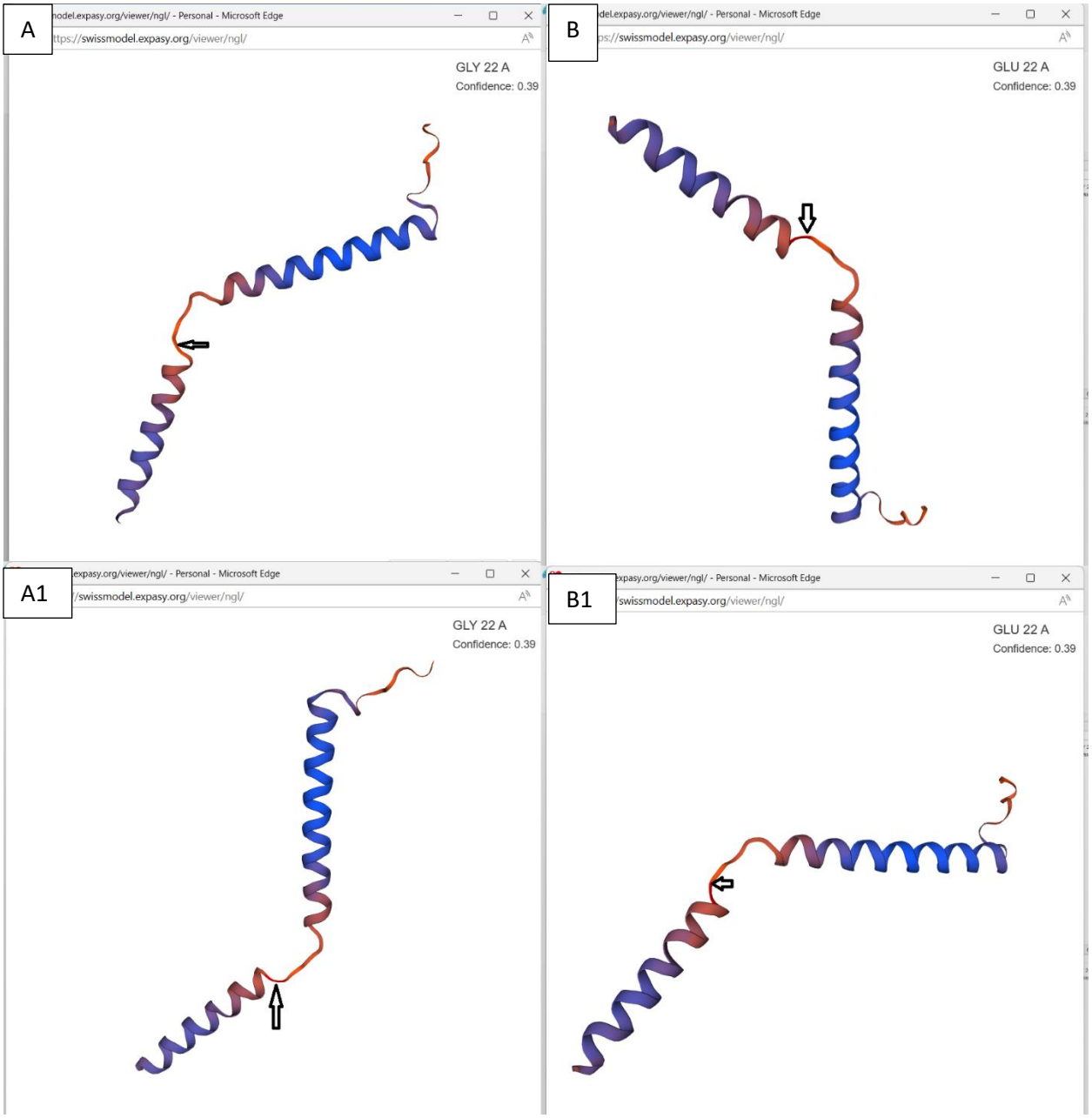

Figure (4.7): Substitution of amino acids of *TNF-alpha* gene (A and A1 normal 3D of secondary structure ribbon model, B and B1 abnormal 3D of secondary structure ribbon model. According to Swiss Model software.

4.7.2. Detection of IFN-γ gene polymorphisms

In the present study, the *IFNG* genetic sequences in chromosome 12. This gene encodes for the interferon-gamma that is involved in several activities related to the antiviral, antimicrobial, and antitumor responses by activating effector immune cells and antigen presentation (https://www.uniprot.org/uniprot/P01579). The sequencing reactions indicated the exact identity of this genetic fragment after performing NCBI blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Concerning the currently investigated 522 bp amplicons of this gene, the NCBI BLASTn engine showed about 99.5% sequence similarities between the sequenced samples and the intended reference target sequences, which completely cover the exon 4 sequences for the interferon-gamma according to the previous annotations of the human genome (GenBank acc. NG_015840.1). By comparing the observed DNA sequences of these investigated samples with the retrieved DNA sequences (GenBank acc. NG_015840.1). However, this SNP was detected in homozygous A/A and C/C status, and a heterozygous C/A was observed in the investigated sample**.**

The alignment results of the 522 bp samples revealed the presence of only one variation in some of the analyzed samples compared with the referring reference DNA sequences. A highly interesting nucleic acid polymorphism (SNP) was detected in this study in the investigated samples, in which Cytosine was replaced with Adenine in the position of the amplified fragment, namely 209C>A.

Figure (4.8): The Alignment Results of the *IFNG* Gene according to BioEdit 7.2 software.

4.7.2.1. Genotyping of IFN-γ Polymorphism

To summarize the results obtained from the sequenced 522 bp fragments, the detailed positions of the observed variations are described in the NCBI reference sequences, as shown in Table (4.11).

The present results showed that DNA polymorphism distribution was according to CC; CA and AA were 44%, 34%, and 22%, respectively, in patients with brain tumors and 72%; 12% and 16%, respectively in The control group. There are significant statistical differences ($p< 0.05$) between different groups according to genotyping of IFN-γ Table (4.11).

Table (4.11). Genotyping of IFN-γ gene (522 bp) gene in comparison with the NCBI referring sequences (GenBank acc. no. NC_000012.12).

IFN-γ	Brain tumors No. (%)	Control No. (%)	Chi-square	p- value	Odd ratio (CI95)
CC	22 44%	18 72%	Reference		
CA	17 34%	3 12%	5.72	0.022*	1.21 (0.45-2.85)
AA	11 22%	4 16%	1.52	0.21	0.44 (0.12-1.63)
Allele					
C	61	39			
A	39	11	4.33	0.037*	1.44 (0.20-1.96)

After that, the sample sequences were submitted in NCBI, and the accession number of nucleotide sequences of IFN-γ gene as new recording as shown appendix (2): Bank It 2617649 ; OP326723 ; OP326724; OP326725; OP326726; OP326727; OP326728 ; OP326729 ; OP326730 ; OP326731.

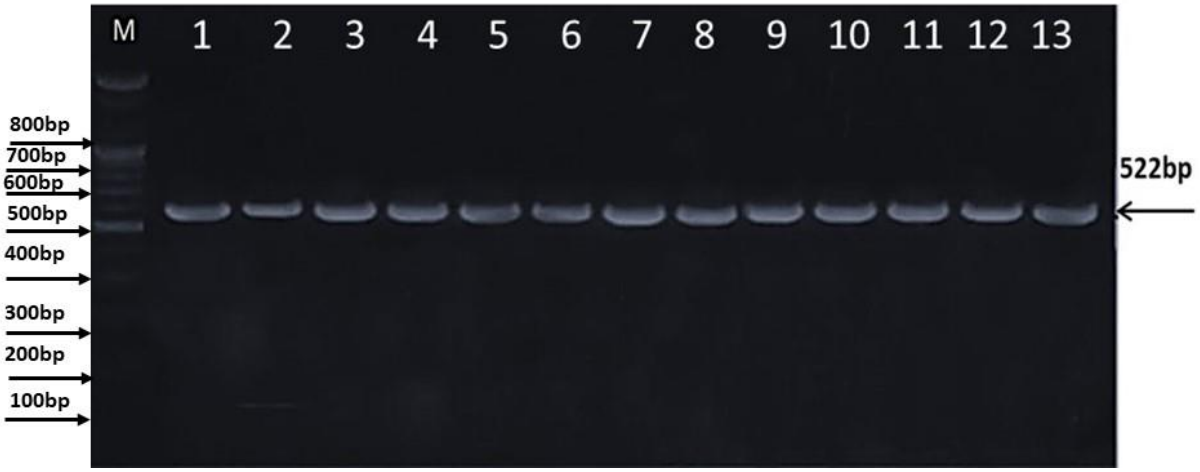

Figure (4.9): Gel electrophoresis of an amplified product pattern of *IFN-γ* gene, with conditions, 1.5% agarose, 75 V, for 1h, 5 µl in each well stained with a red safe solution, Lanes (1-13) were Positive.

4.7.3. Detection of KIRs gene polymorphisms

In the present study, the *KIR2DS2* genetic sequences in chromosome 19. This gene encodes for the killer cell immunoglobulin-like receptor involved in several activities related to inhibiting NK cell's activity to prevent cell lysis (https://www.uniprot.org/uniprot/ P43629). The sequencing reactions indicated the exact identity of this genetic fragment after performing NCBI blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Concerning the currently investigated 530 bp amplicons of this gene, the NCBI BLASTn engine showed about 99.5% sequence similarities between the sequenced samples and the intended reference target sequences, which completely cover the

exon-4-sequences for the killer cell immunoglobulin-like receptor according to the previous annotations of the human genome (GenBank acc. LT996868.1). By comparing the observed DNA sequences of these investigated samples with the retrieved DNA sequences (GenBank acc. LT996868.1). However, this SNP was detected in homozygous A/A and T/T status, and a heterozygous A/T was observed in the investigated sample**.**

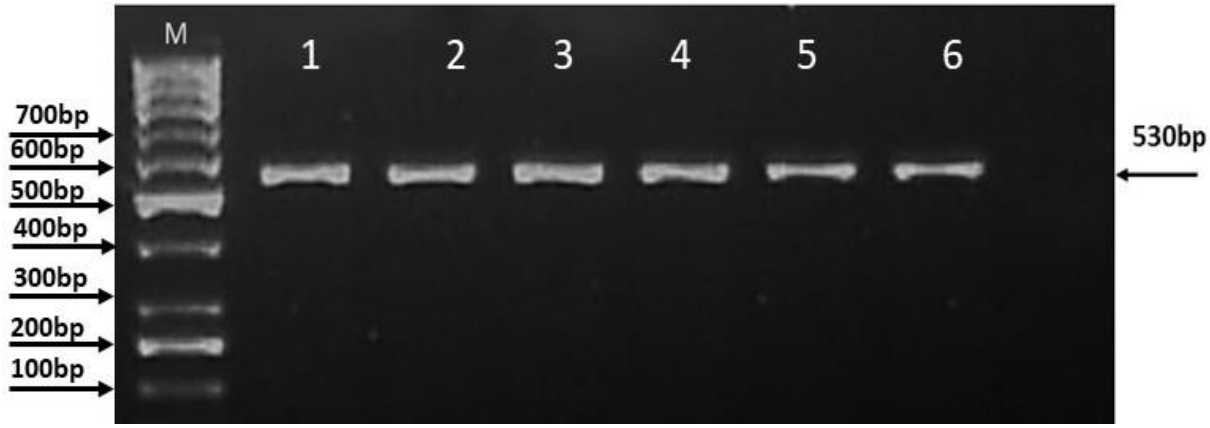

Figure (4.10): PCR amplification with specific primers for *KIR* gene, with conditions, 1.5% agarose, 75 V, for 1h, 5 µl in each well stained with a red safe solution, Lanes (1-6) were Positive.

The alignment results of the 530 bp samples revealed the presence of only one variation in some of the analyzed samples in comparison with the referring reference DNA sequences. In this variant, the nucleic acid Adenine was replaced with Thymine in position 225 of the amplified fragment, namely 225A>T.

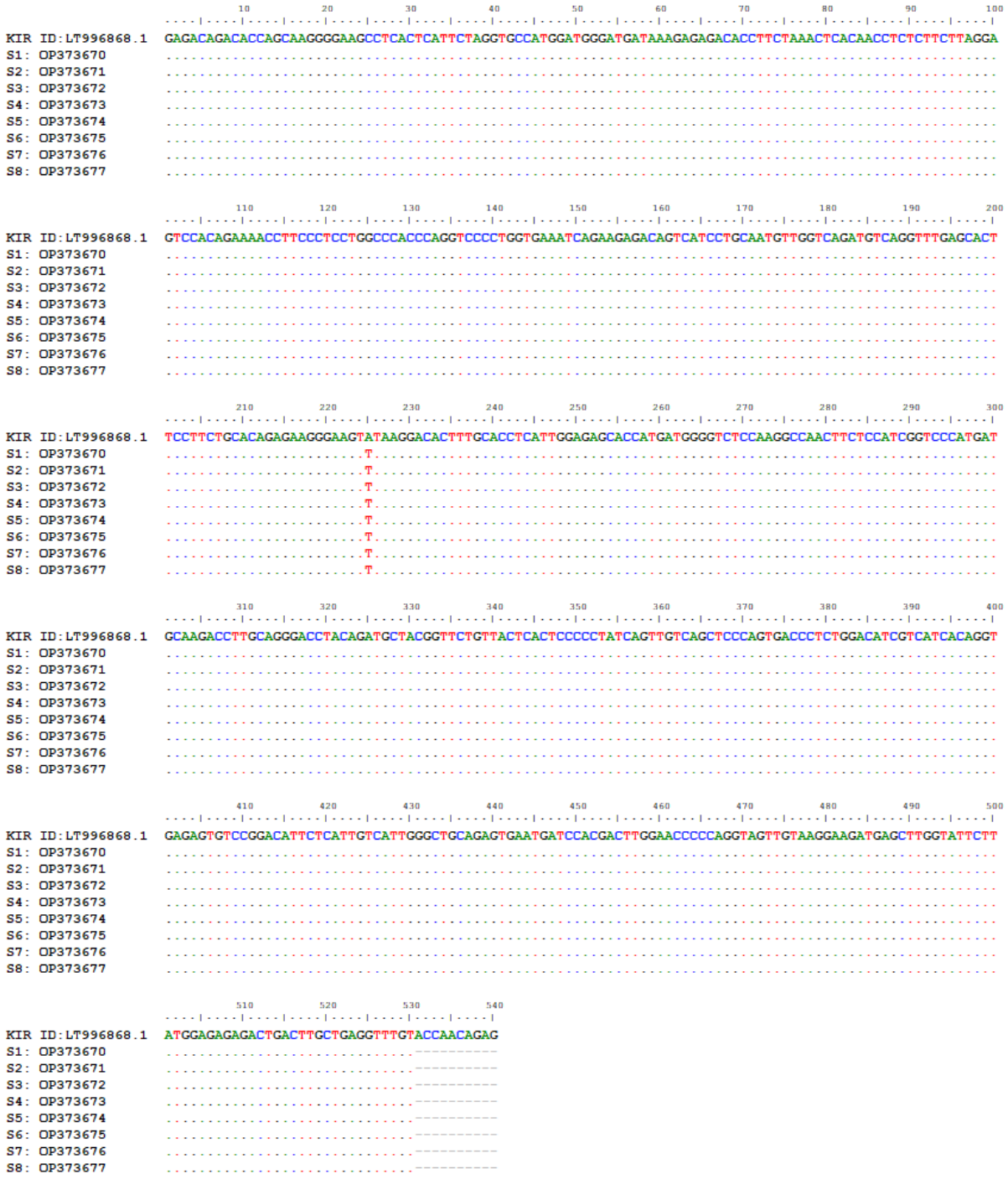

Figure (4.11): The Alignment Results of the *KIR* Gene according to BioEdit 7.2 software.

4.7.3.1. Genotyping of KIRs Polymorphism

To summarize the results obtained from the sequenced 530 bp fragments, the detailed positions of the observed variations are described in the NCBI reference sequences, as shown in Table (4.12).

The results showed that DNA polymorphism distribution was according to TT; AT and AA, which were 38%, 32%, and 30%, respectively, in patients with brain tumors and 76%; 8%, and 16%, respectively in the control group. Statistical comparison of these polymorphisms revealed significant differences ($p< 0.05$) in Table (4.12).

Table (4.12). Genotyping of KIR2DS2 gene (530 bp) comparison with the NCBI referring sequences (GenBank acc. no. NC_000019.10).

KIR2DS2	Brain tumors No. (%)	Control No. (%)	Chi-square	p- value	Odd ratio (CI95)
TT	19 38%	19 76%	Reference		
AT	16 32%	2 8%	7.88	0.005*	1.12 (0.52-2.62)
AA	15 30%	4 16%	4.41	0.036*	1.26 (0.07-2.95)
Allele					
T	54	40			
A	46	10	9.63	0.002*	1.29 (0.13-1.65)

The sample sequences were submitted to NCBI, and the accession number of nucleotide sequences of the KIR gene as a new recording as shown in Appendix (3). BankIt2619196 OP373670; OP373671; OP373672; OP373673; OP373674; OP373675; OP373676; OP373677.

4.7.3.2. Protein folding of *KIR2DS2* gene

When translating the DNA sequence using by Bio Edit program version 7.2.5 according to the reference sequence alignment of the human *KIR2DS2* gene ID: NP_056952.2. The results appeared to change an amino acid residue of *KIR2DS2* protein as shown in figure (4.12). These amino acid substitutions were phenylalanine to tyrosine in position 66 that may affect or modified the tertiary structure of the protein, subsequently, the function may be effected figures (4.13).

Figure (4.12): Protein sequences alignment of the genotyped sample with their corresponding reference sequences of the 304 amino acids of the *KIR2DS2* gene. The symbol "LT996868.1" refers to the NCBI referring sequence. The "S" refers to the genotyped sample. By reviewing the dbSNP details of the identified 225A>T , this SNP caused an amino acid substitution of phenylalanine to tyrosine in position 66.

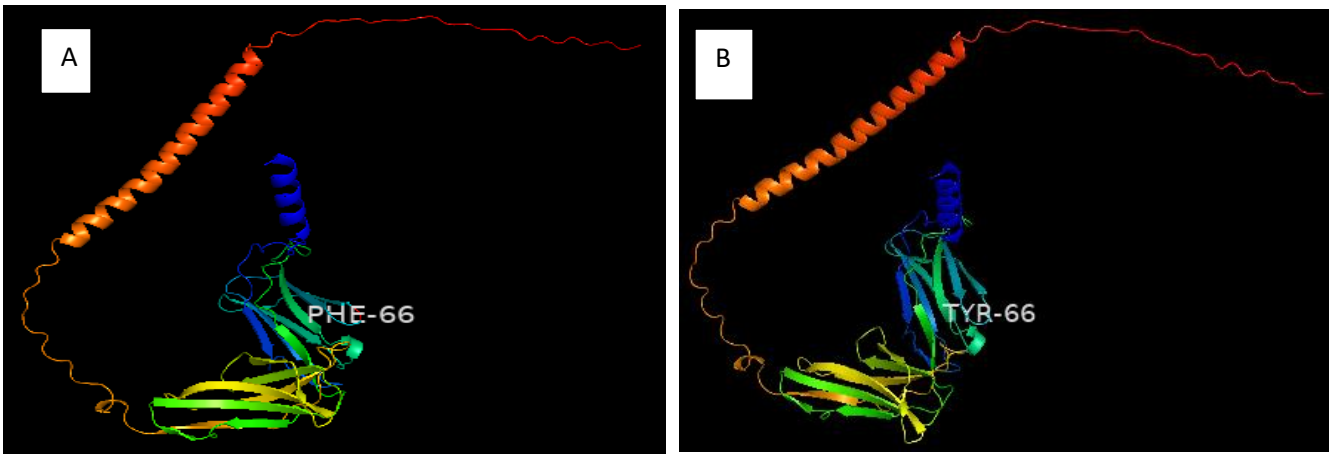

Figure (4.13): Substitution of amino acids of *KIR2DS2* gene (A normal 3D of secondary structure ribbon model, B abnormal 3D of secondary structure ribbon model. According Pymol l 2.5.2 program.

4.7.4. Detection of IL-1Ra gene polymorphisms

In the present study, the *IL1RN* genetic sequences in chromosome 2. This gene encodes for the interleukin-1 receptor antagonist that inhibits the interleukin-1 activity to prevent its signaling potentials (https://www.uniprot.org/uniprot/P18510). The sequencing reactions indicated the exact identity of this genetic fragment after performing NCBI blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Concerning the currently investigated 477 bp amplicons of this gene, the NCBI BLASTn engine showed about 99.5% sequence similarities between the sequenced samples and the intended reference target sequences, which completely cover exon-1 sequences for the interleukin 24A according to the previous annotations of the human genome (GenBank acc. NG_021240.1). By comparing the observed DNA sequences of these investigated samples with the retrieved DNA sequences (GenBank acc. NG_021240.1).

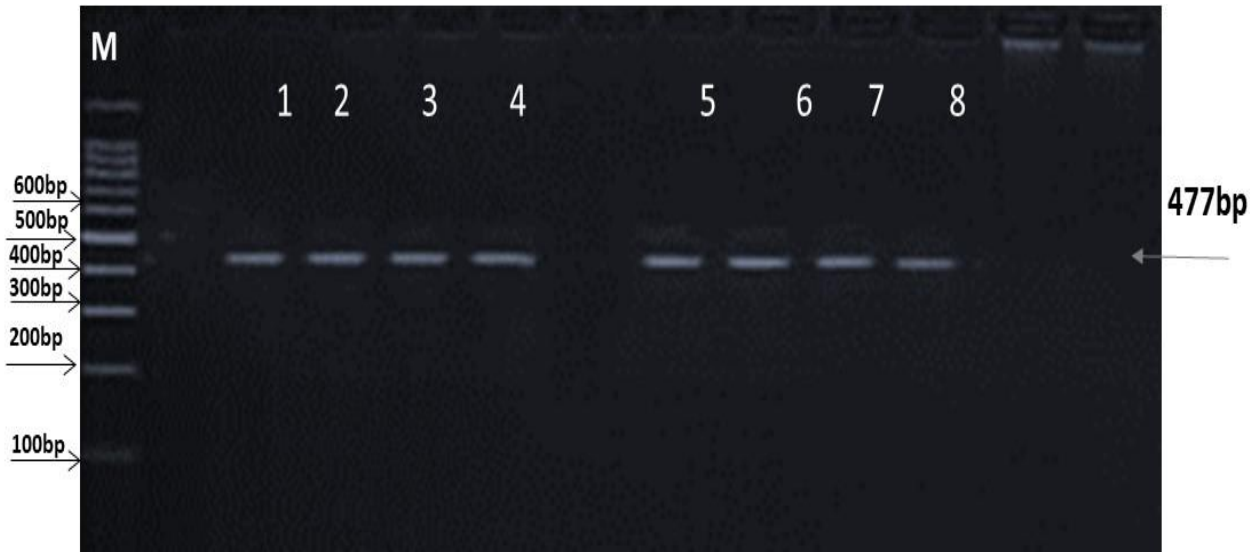

Figure (4.14): PCR amplification with specific primers for *IL1Ra* gene, with conditions, 1.5% agarose, 75 V, for 1h, 5 µl in each well stained with a red safe solution, Lanes (1-11) were Positive.

The alignment results of the 477 bp samples revealed the presence of five variations in some of the analyzed samples in comparison with the referring reference DNA sequences. Five highly interesting nucleic acid polymorphisms (SNP)s were detected in this study in the investigated samples. Both in first and second variant observed, the nucleic acid Guanine was replaced with Adenine in positions 173 and 254 of the amplified fragment, namely G>A. In the third variant, the nucleic acid Adenine was replaced with Guanine in position 310 of the amplified fragment, namely 310 A>G. In the fourth variant, the nucleic acid Guanine was replaced with Cytosine in position 329 of the amplified fragment, namely 329 G>C. In the fifth variant, the nucleic acid Thymine was replaced with Cytosine in position 376 of the amplified fragment, namely 376 T>C.

Figure (4.15): The Alignment Results of the *IL1Ra* Gene according to BioEdit 7.2 software.

4.6.4.1. Genotyping of IL-1Ra Polymorphism

To summarize the results obtained from the sequenced 477 bp fragments, the detailed positions of the observed variations are described in the NCBI reference sequences, as shown in Table (4.13).

The results of **rs2234679** showed that DNA polymorphism distribution was according to CC; GC and GG were 40%; 15% and 30%, respectively, in patients with brain tumors and 68%, 12%, and 20%, respectively in the control group. While The results of **rs16065** showed that DNA polymorphism distribution was according to TT; TC and CC were 54%, 16%, and 30%, respectively, in patients with brain tumors and 60%; 12% and 28%, respectively in the control group. The difference in frequency of genotype distribution of the polymorphism between patients and control groups was statistically non-significant as in Table (4-13).

Table (4.13). Genotyping of IL-1Ra gene (477 bp) comparison with the NCBI referring sequences (GenBank acc. no. NC_000019.10).

IL1Ra	Brain tumors No. (%)	Control No. (%)	Chi-square	p- value	Odd ratio (CI95)
rs2234679					
CC	20 (40%)	17 (68%)	Reference		
GC	15 (30%)	3 (12%)	4.48	0.034*	0.23(0.05-0.95)
GG	15 (30%)	5 (20%)	2.40	0.12	0.39(0.11-1.30)
Allele					
C	55	37			
G	45	13	5.07	0.024*	0.42(0.20-0.90)
rs16065					
TT	27 (54%)	15 (60%)	Reference		
TC	8 (16%)	3 (12%)	0.27	0.59	0.67(0.15-2.93)
CC	15 (30%)	7 (28%)	0.09	0.75	0.84(0.28-2.51)
Allele					
T	62	33			
C	38	17	0.58	0.44	1.32(0.64-2.69)

The samples sequences were submitted in NCBI, and the accession number of nucleotide sequences of IL1Ra gene, as a new recording as shown Appendix (4). BankIt2618097; OP351527; OP351528; OP351529; OP351530; OP351531; OP351532; OP351533; OP351534; OP351535; OP351536.

4.7.5. Detection of *IFNL2* (or *IL-28A*) gene polymorphisms

In the present study, the *IFNL2* (or *IL-28A*) genetic sequences in chromosome 19. This gene encodes for the interleukin 28A and is involved in several activities related to antiviral, antitumor, and immunomodulatory activities (https://www.uniprot.org/uniprot/Q8IZJ0). The sequencing reactions indicated the exact identity of this genetic fragment after performing NCBI blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Concerning the currently investigated 604 bp amplicons of this gene, the NCBI BLASTn engine showed about 99.5% sequence similarities between the sequenced samples and the intended reference target sequences, which completely cover Two exonic sequences for the interleukin 24A according to the previous annotations of the human genome (GenBank acc. DQ126336.2). By comparing the observed DNA sequences of these investigated samples with the retrieved DNA sequences (GenBank acc. DQ126336.2). However, this SNP was detected in homozygous GG and CC status, and a heterozygous GC was observed in the investigated sample**.**

The alignment results of the 604 bp samples revealed the presence only one variation in some of the analyzed samples in comparison with the referring reference DNA sequences. Highly highly interesting nucleic acid polymorphisms (SNPs) were detected in this study in the investigated samples. The nucleic acid Guanine was replaced with Cytosine in position 350 of the amplified fragment, namely 350G>C.

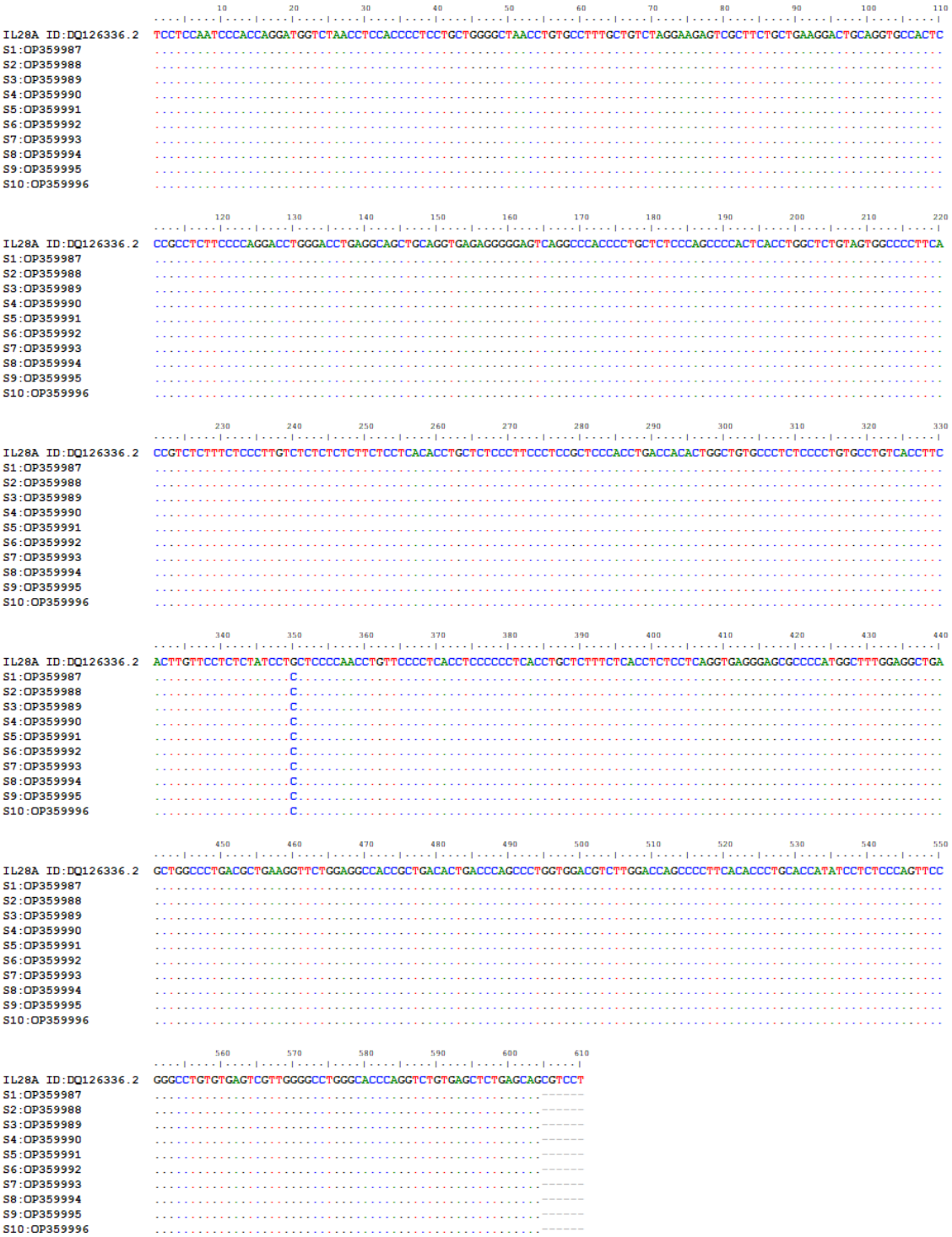

Figure (4.16): The Alignment Results of the *IL28A* Gene according to BioEdit 7.2 software.

4.7.5.1. Genotyping of *IFNL2* (or *IL-28A*) Polymorphism

To summarize the results obtained from the sequenced 604 bp fragments, the detailed positions of the observed variations are described in the NCBI reference sequences, as shown in Table (4.15).

The results showed that DNA polymorphism distribution was according to GG; GC and CC were 46%; 20% and 34%, respectively in patients with brain tumors and 64%; 20% and 16%, respectively in The control group. The statistical analysis of ***IFNL2* (or *IL-28A*)** polymorphism shows non-significant differences ($p<0.05$) between patients with brain tumor groups as well as control groups Table (4-14).

Table (4.14). Genotyping of *IFNL2* (or *IL-28A*) gene (604 bp) comparison with the NCBI referring sequences (GenBank acc. no. NC_000019.10).

IL-28A	Brain tumors No. (%)	Control No. (%)	Chi-square	p- value	Odd ratio (CI95)
GG	23 46%	16 64%	Reference		
GC	10 20%	5 20%	0.20	0.60	0.71(0.20-2.50)
CC	17 34%	4 16%	2.96	0.08	0.33(0.09-1.19)
Allele					
G	56	37			
C	44	13	2.29	0.13	0.58(0.29-1.17)

After that, the sample sequences were submitted in NCBI, and the accession number of nucleotide sequences of IL28A gene as new recording as shown in Appendix (5): BankIt2618865: OP359987; OP359988; OP359989; OP359990; OP359991; OP359992; OP359993; OP359994; OP359995; OP359996.

Figure (4.17): Gel electrophoresis of amplified product patterns of *IL28A* gene, with conditions, 1.5% agarose, 75 V, 20 mA for 1h (5 µl in each well), stained with a red safe solution Lanes (1-12) were Positive.

4.8. Evaluation of the Serum Level of TNF-alpha (TNF-α); Interferon-gamma (IFN-γ); Killer Immunoglobulin-like Receptors (KIRs); Interleukin 1 R a (IL-1Ra) and IL-28 By ELISA among Study Population:

4.8.1. Evaluation the serum level of TNF- alpha (TNF-α) in patients with Brain Tumors and AHC.

Table (4.15) shows the mean of serum level TNF-α for AHC and patients with brain tumor groups. Statistically, a significant difference ($p<0.05$) was found on comparing the mean of serum level of TNF-α among these study groups.

Table (4.15): The serum level of TNF-α by ELISA in patients with brain tumor and AHC

Age group	Brain tumors	AHC	*P value*
	Mean± SE	**Mean± SE**	
2-18	15.61±0.6	11.36±0.3	***P*<0.05 (0.03)** *
19-35	12.13±0.4	6.48±0.1	
36-52	11.17±0.3	7.13±0.2	
53-69	14.26±0.7	5.31±0.25	
70-85	20.08±0.9	9.73±0.7	

***Statistical significant**

AHC: Apparently health control

4.8.2. Estimation the serum level of Interferon-gamma (IFN-γ) Among Study Groups.

Table (4.16) was shown the mean of serum level IFN-γ for AHC and patients with brain tumor groups. Statistically, a significant difference ($p<0.05$) was found on comparing the mean of serum level IFN-γ among these study groups.

Table (4.16): The serum level of IFN-γ by ELISA in patients with brain tumor and AHC

Age group	Brain tumors	AHC	*P value*
	Mean± SE	**Mean± SE**	
2-18	14.63±2.01	7.32±0.92	***P*<0.05 (0.03)** *
19-35	10.13±1.5	6.23±0.84	
36-52	11.67±0.9	3.79±0.4	
53-69	12.98±1.6	3.1±0.9	
70-85	18.1±1.9	5.81±0.84	

***Statistical significant**

AHC: Apparently health control

4.8.3. Determination of Killer Immunoglobulin-like Receptors (KIRs) Among Study Groups

Table (4.17) shows the mean of serum level of KIRs for AHC and patients with brain tumor groups. Statistically, a significant difference ($p<0.05$) was found on comparing the mean of serum level of KIRs among these study groups.

Table (4.17): The serum level of KIRs by ELISA for patients with brain tumor and AHC

Age group	Brain tumors	AHC	*P value*
	Mean± SE	**Mean± SE**	
2-18	14.25±0.5	6.37±0.76	***P*<0.05 (0.03)** *
19-35	9.11±0.4	3.43±0.52	
36-52	8.23±0.6	4.51±0.31	
53-69	10.40±0.5	2.78±0.3	
70-85	11.82±0.7	4.61±0.91	

***Statistical significant**

AHC: Apparently health control

4.8.4. Estimation the level of Interleukin 1 R a (IL-1Ra) Among Study Groups.

Table 4-18 was shown the mean of serum level of IL-1Ra for AHC and patients with brain tumors groups. Statistically, a significant difference ($p<0.05$) was found in comparing the mean of serum level of IL-1Ra among these study groups (Table 4.19).

Table (4.18): The serum level of IL-1Ra concentration by ELISA for patients with brain tumor and AHC

Age group	Brain tumors	AHC	*P value*
	Mean± SE	**Mean± SE**	
2-18	13.77±1.2	7.32±0.89	***P*<0.05 (0.0)***
19-35	9.94±0.6	2.89±0.75	
36-52	11.67±0.7	4.51±0.39	
53-69	10.93±1.4	3.92±0.73	
70-85	11.82±0.7	4.61±0.91	

***Statistical significant**

AHC: Apparently health control

4.8.4. Evaluation the serum level of Interleukin -28 (IL-28) Among Study Groups.

Table (4.19) showed that the mean serum level of IL-28 for AHC and patients with brain tumors groups. Statistically, a non-significant difference ($p<0.05$) was found in comparing the mean of serum level of IL-28 among these study groups.

Table (4.19): The serum level of IL-28 by ELISA for patients with brain tumor and AHC

Age group	Brain tumors	AHC	*P value*
	Mean± SE	Mean± SE	
2-18	11.79±0.71	8.12±0.95	***P*<0.05 (0.07)**
19-35	10.61±1.09	5.92±0.68	
36-52	8.67±0.6	4.76±0.85	
53-69	7.91±1.4	6.29±0.78	
70-85	13.67±0.9	9.61±0.89	

AHC: Apparently health control

4.9. Spearman's Rho Statistical Testing to Evaluate the Studied Markers in Study Population Groups.

A strong positive relationship (with highly significant correlation) was found between HTLV-1 and SNP Tumor necrosis-factor alpha (TNF-α); Interferon-gamma (IFN-γ); Killer immunoglobulin-like Receptors (KIRs); Interleukin 1 R a (IL-1Ra) in brain tumors (r =0.739*, P =0.04; r =0.675 * P =0.04; r =0.834*, P =0.03; r =0.969**; P =0.02), respectively.

On the other hand, the correlation was statistically non-significant between HTLV-1 and IL-28 in brain tumors (r = 0.159, P =0.57). Similarity non-significant

between HTLV-1 and sex as well as age groups in brain tumors (r =0.279, P =0.6 and r =0.123, P =0.5, respectively)

However, there was no significant correlation among types of brain tumors and SNPs of Tumor necrosis-factor alpha (TNF-α); Interferon-gamma (IFN-γ); Killer Immunoglobulin-like Receptors (KIRs); Interleukin 1 R a (IL-1Ra) and IL-28 in brain tumors. (r =0.323, P =0.5; r =0.336, P =0.5; r=0.432, P =0.5; r =0.439, P =0.5 and r =0.496, P=0.5), respectively as illustrated in Table (4.20)

Table (4.20): Spearman's Rho Statistical Testing of Age, Types of Brain tumors, HTLV-1 and SNPs of TNF-α; IFN-γ; KIRs; IL-1Ra and IL-28 to evaluate the Studied Markers in patients with Brain tumors

Spearman's rho		HTLV-1	TNF-α	IFN-γ	KIRs	IL-1Ra	IL-28
Age	R	0.123	**0.986	**0.986	**0.678	**0.721	**0.567
	P	0.5	0.007	0.02	0.03	0.03	0.04
Types of Brain tumors	R	0.788**	0.323	0.336	0.432	0.439	0.496
	P	0.009	0.5	0.5	0.5	0.5	0.5
Grade	R	0.279	0.763*	0.655*	0.743*	0.674*	0.594*
	P	0.6	0.02	0.04	0.03	0.04	0.04
HTLV-1	R		0.739*	0.675 *	0.834*	0.969**	0.159
	P		0.04	0.04	0.03	0.02	0.57

***Statistical significant**

**** Highly statistical significant**

Chapter Five

Discussion

5. Discussion

5.1. Brain Tumors

Malignant brain tumors remain incurable diseases. Although much effort has been devoted to improve patient outcomes, multiple factors such as the high tumor heterogeneity, the strong tumor-induced immunosuppressive microenvironment, and the low mutational burden make the treatment of these tumors especially challenging **(**Sostoa *et al*. 2020).

5.1.1. Distribution of Patients with Brain Tumors and Control Groups According to Their Age

In the current study, this research work provided an analysis of histology-specific statistical analysis of enrolled brain tumors from a group of patients in Iraq, where the age distribution of studied patients revealed a wide age- range from 2 to 85 years with a mean age of 50.7 + 10.4 years as compared to 47.9 + 12.6 years for their control counterparts and included the patients with brain and CNS tumors in the range described for the adolescents and young adults (AYA) group of patients as well as those older than them. The highest male frequency (12) was found in the 53–69 year age group, whereas the highest female frequency (10) was found in the 70–85 year age group. These results are in agreement with Hamid and Khan (2020), who found the maximum percentage were from the age group of 51-60 years (41%) followed by 41-50 as well as >60 years (20%). While minimum percentage were in the age group of 10-20 years (2%) followed by 21-30 years (4%).

Brain tumors can start at any age. The incidence of brain tumors overall increases with age. The risk of brain tumors is greatest in those aged between 85 and 89 years (Ostrom *et al*. 2019). The most common overall brain tumor

histology also varied from both those aged 0-14 years and more than 40 years (Stiller *et al*. 2019).

Cancer survivals have improved overall, and especially in those diagnosed at younger ages who have significantly longer survivals, yet, those in AYA have not experienced such increased survivals or, even worse in some cases, over age 40 years (Ostrom *et al*. 2016). Most common CNS tumors, relative to all cancers in specific age groups, occurred at a rate of 4.4% in 15-39 years, 32.4% in 0-14 years, and 2.2% in those more than 40 years (Rostgaard *et al*. 2019).

Heng *et al*. (2023) consistently the incidence of brain tumors is closely related to age. Most brain tumors are being diagnosed in adults, with the highest incidences in patients aged 51 to 60 years (26%). Also, deal with Othman *et al*. (2020) and Goh *et al*. (2014).

Furthermore, a study by Victor *et al*. (2011) showed that about 60% of patients with brain metastasis are aged between 50 and 70 years. Porter *et al*. (2010) 7.5% of brain tumors have presented in those under 20 years of age, and the majority (69.3%) of those were malignant, as opposed to brain tumors in adults, where (37.5%) were malignant.

Notably, the profiles of strengths and deficiencies in executive functions were nearly the same when comparing only 9 to 13-year-old individuals, despite the fact that the brain disorder groups differed in age and other age-related variables. These findings suggest that age at injury is not the only factor influencing the variations in executive function patterns displayed by children with various developmental brain disorders (Araujo *et al*., 2017)**.**

The reasons for the difference between ages at presentation in subjects from the various regions are not clear. Whether this represents a true geographic/ethnic

difference or merely the result of varying referral patterns is difficult to ascertain. These differences in environmental and geographical risk factors affect each study groups making brain tumors affecting relatively middle age groups in Iraq and neighboring countries.

5.1.2. Distribution of Patients with Brain Tumors and Control Groups According to Their Sex

Malignant brain tumors (male: 8.28/100,000, female: 5.98/100,000) were more easily seen in men than women, and the opposite was true for nonmalignant tumors (male: 13.07/100,000, female: 20.97/100,000) (Ostrom *et al*., 2021).

The present results showed that the highest male frequency 12 was found in the 53–69 year age group, whereas the highest female frequency 10 was found in the 70–85 year age group. These results are compatible with Hamid and Khan (2020), who revealed that females and males comprised 56% and 44% of the subjects, respectively. Hence, females were comparatively more as compared to males, though statistically insignificant.

Also, the current results were consistent with Saha *et al*. (2013) in their study which found that there was an almost equal distribution of males versus females (52.78% vs. 47.22%).

In contrast, current results, inconsistent with Heng *et al*. (2023), reveal that males constituted 42.6% of the cases, whereas 57.4% of them were female. The result is consistent with the other epidemiology studies on brain tumors in the United States (CBTRUS Statistical Report in 2019) and Malaysia.

In addition, Ravshanov (2022) reported that according to the distribution, there were 26 (35.6%) male and 47 (64.4%) female patients. Ghosh *et al*. (2017), in their

study, revealed similar results too, i.e., female preponderance was seen constituting 68.69% whereas males constituted 31.31%.

For malignant brain tumors, gender differences gradually became apparent in adults aged ≥40 years. In adults aged ≥45 years, the gender differences were greatest, with 30% lower rates in females than males (ratio of female to male incidence rate, 0.69; 95% CI: 0.68–0.70) (Miller *et al*. 2021).

Males showed higher age-standardized rates of incidence, mortality, and DALY than females across the age groups, which was consistent with the results of previous studies (Gigineishvili *et al*., 2013; Ostrom *et al*., 2013).

Such differences may have been due to genetic differences, lifestyle differences, and differences in medical attitudes and habits.

5.1.3. The Grading of the Studied Brain Tumors:

Brain tumors are one of the most common causes of death worldwide, and they account for about 3% of the mortality of all cancers and allied deaths. Worldwide, an estimated 251,329 people died from primary brain cancer and central nervous system (CNS) tumors in 2020. Unlike other tumors, they are not staged, and WHO has assigned a Grade (I through IV) to predict their outcome (Almatroudi, 2022).

In this study, grade I was found in 37 cases (49.3%) of brain tumor cases (20 males and 17 females), while grade II was found in 17 cases (22.7%) (9 males and 8 females), grade VI were seen in 13 cases which were (17.3%) (7 males and 6 females); finally, grade III were seen in 8 cases only (10.7%) of brain tumor group (5 males and 3 females)**.** These results are in agreement with Mohammed *et al*. (2019), revealing that 61 (87.1%) cases of Grade I meningiomas, 6 (8.6%) cases of Grade II, and 3 (4.3%) cases of Grade III.

Salari, *et al.*, (2022) reported that patients with astrocytoma (grade II) have a 5-year survival rate of 66%, while the 10-year survival rate of patients with low-grade astrocytomas was 11%.

Garcia, *et al*. (2019) reported that the tumors were as World Health Organization (WHO) grade I for 55.9% of the patients, grade II for 5.9%, grade III for 4.4%, grade IV for 24.3%, and grade unknown or not applicable for 9.4%.

Two benign tumors, pilocytic astrocytoma (grade I) and low-grade astrocytoma (grade II), arise from astrocytes, and over time, they may progress to a higher grade (Chourmouzi *et al*., 2014). The astrocytomas of grade I and II are usually located in the frontal lobe of the brain and most often affect those patients in their 3rd or 4th decade of age (Dong *et al*., 2016).

5.1.4. The Typing of the Studied Brain Tumors

Conventionally, brain tumors are classified according to the cell of origin or the site of origin, such as neuroepithelial origin (including astrocytic tumors, oligodendroglial tumors, oligoastrocytic tumors, ependymal tumors, choroid plexus tumors, neuronal and mixed neuronal-glial tumors, pineal tumors, and embryonal tumors), tumors of cranial nerves, tumors of the meninges, lymphomas and hematopoietic neoplasms, germ cell tumors, tumors of the sellar region, and metastases. The WHO classification of brain tumors (2016) introduced a greater reliance on molecular markers (Louis *et al*., 2016).

The present study included 21 cases of brain tumors as Pilocytic Astrocytoma (28 %) (11 males and 10 cases were females), 15 cases as Meningotheliomatous Meningioma (20%) (8 males and 7 females), 13 cases as Glioblastoma Multiforme and Diffuse Fibrillary Astrocytoma (17.3%) (7 males and 6 females) for each, 6 cases as Anaplastic Oligodendroglioma (8%) (3males and 3 females), 4 cases as Atypical Meningioma (5.4 %) (3 males and 1 female), and 3 cases as Transitional Meningioma (4 %) (2 males and 1 female)**.** These results are consistent with Girardi *et al*. (2023); tumors originating in the brain are rare. During 2010–2014, the global range in age-standardized 5-year survival within each histology group was remarkably wide: in the range of 4%–17% for glioblastoma, 20%– 38% for diffuse and anaplastic astrocytoma, and 32%–69% for oligodendroglioma.

Glioblastoma multiforme (GBM) is the most common adult brain neoplasm in the 5^{th} and 6^{th} decades. It is a rapidly growing and highly malignant one, as well as having a grade IV astrocytoma classification (Geteneh *et al*., 2022). Males are affected more frequently than females, and it is frequently found in the frontal lobe of the brain (Grech *et al*., 2020).

Mohammed *et al*. (2019) revealed that the most common diagnosis among females was meningiomas (40%); among males, it was astrocytic tumors (42.6%).

Ravshanov (2022) reported that according to the histological classification adopted by the WHO in 2007, there are 15 subtypes of meningiomas divided into 3 grades based on their malignancy.

Previous studies have reported that the incidence of anaplastic astrocytoma and glioblastoma increased with age, peaking in the 75–84 year age group (McNeill, 2016).

The incidences of GBM, diffuse astrocytoma and other gliomas were higher in males than in females in the same age group. For non-malignant brain tumors, gender differences gradually became apparent in adults aged ≥20 years. The gender difference was mainly in meningiomas and pituitary tumors. Especially for non-malignant meningiomas, the incidence rate of females was about twice that of males in adults aged ≥65 years (Ostrom *et al*., 2019).

5.2. Detection of HTLV-1 in Patients with Brain Tumors by rt-PCR

5.2.1 Extraction of Viral Nucleic Acid from Patients with Brain Tumors

Although causal relationships between brain tumors and infections are difficult to establish, the detection rate of several viruses such as HHV-6, EBV, CMV, and HTLV-1 during disease is an important way to analyze their relationship with the first stage of brain tumors.

By implementing real-time PCR as a diagnostic technique for retroviruses, many more and different viruses are detected, making the performance of these techniques worthwhile. Therefore, the detection of virus particles in brain tumors has made the real-time PCR assay very important because the examination has a high sensitivity and specificity toward diagnosing viral particles in samples. It has now become difficult to determine whether oncogenic retroviruses can cause tumors; in fact, they can be attributed to the types of many viruses that are related to brain disease (Ranjith *et al*., 2023). The Ct-value is a semi-quantitative measure of the viral load in a specimen. It is inversely correlated to the amount of virus; hence, the lower the Ct-value, the higher the viral load (Phillip *et al*., 2009).

Out of 75 brain tumor specimens involved in this study, 50 (66.7%) were found to have a viral infection. Twenty-five brain tumor specimens without a viral genome were detected in this study, accounting for 33.3% of the total number of

patients. While only 5 (6.6%) of the control group have viral genomes as shown in Table (4.6).

It may be possible that the quantity or the physical status of viral DNA in the tissue of patients does not permit its easy detection and requires the use of the more sensitive and specific nested PCR technique. In addition, it remains unclear whether tissues, as well as blood specimens, are optimal for the detection of most oncovirus by molecular techniques such as multiplex PCR or RT-PCR (Ji et *al*., 2023).

5.2.2. Detection of HTLV-1 by RT.PCR.

It is generally thought that the optimal specimen type for detecting HTLV-1 is tissue as well as blood specimens, although this may not be true for all HTLV-1 detection techniques. Given the complexity and invasive nature of the procedure used to acquire blood specimens have emerged as an alternative specimen type. Tissues, as well as blood specimens, were shown to be superior or equivalent to specimens for RT-PCR or PCR in many studies (Ji et *al*., 2023).

Qualitative RT-PCR is one of the most important molecular techniques that have been applied for the detection of HTLVs. This test has enabled rapid, accurate, and simultaneous detection of retroviruses with enhanced sensitivity and specificity (Rocha-Junior *et al*., 2022; Ji *et al*., 2023).

Previous studies have reported viruses in the CSF of patients with simultaneous bacterial meningitis, including human enterovirus and human herpes viruses, mostly EBV, while Rhinovirus had been detected in a child with *Haemophilus influenzae* type b meningitis. A previous study had suggested that viruses might predispose the child to bacterial meningitis, where such viral infections may enhance bacterial adherence via disrupting respiratory epithelium or increasing

bacterial cellular interaction, and their concomitantly detection (bacterial and viral agents in such cases) proposed simultaneously occurred infections, or very close timing of co- occurrence (Pelkonen *et al*., 2012; Stafford *et al*., 2016).

The positive results according to qRT-PCR showed 42 % (21 out of 50 cases) as positive while 58% (29 out of 50 cases) as negative. While no positive signals for HTLV-1 in the control group as shown in Table (4.7).

In contrast, Ishida *et al*. (2000), among hospital-based 3,427 subjects in Southern Thailand, one patient with a brain tumor showed positivity in the Western blotting test; however, *HTLV-1* proviral genome was not identified by PCR, suggesting that HTLV-1 is not endemic in the Thai population and that HTLV-1 is not a major public health problem in Thailand because *HTLV-1* rarely causes its associated diseases.

Human T Lymphotropic Virus-1 crosses the blood-brain barrier by migration of HTLV-1-infected lymphocytes in vivo. The ability of HTLV-1 to cause associated neuropathies starts with the virus crossing the blood-brain barrier (BBB), then entering and infecting the cells of the central nervous system (Lotan *et al*. 2012).

HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP), rheumatic syndromes, and HTLV-associated uveitis, among other autoimmune conditions. HAM/TSP is a chronic meningomyelitis of gray and white matter in the spinal cord that presents with spastic paraparesis, impairment of the gait, and autonomic dysfunction of the bowel and bladder (Goncalves *et al*., 2010).

Most HTLV-1-infected individuals remain asymptomatic throughout life, but HTLV-1 has the potential to progress to detrimental neuro-inflammatory diseases in humans, including HTLV-associated myelopathy/tropical spastic paraparesis

(HAM/TSP), rheumatic syndromes, polymyositis, uveitis, Sjogren's syndrome, and systemic lupus erythematosus (SLE) (Goncalves *et al*.,2010; Abolbashari *et al*.,2018).

The ability of HTLV-1 to enter the CNS is a critical event for the development of HAM/TSP, although several reports have shown that HTLV-1 can colonize within cells of the CNS, the precise mechanisms by which the virus traverses the BBB still need to be better understood (Forrester *et al*., 2022).

Enhanced adhesion and transmigration of HTLV-1-infected T lymphocytes with rat brain endothelial cells in vitro has suggested that the trafficking of infected lymphocytes and macrophages across the BBB may be a mechanism of viral entry into the CNS (Romero *et al*., 2000).

HTLV-1 Tax and HBZ are two key proteins involved in ATL oncogenesis. Tax expression is thought to play a role in tumor initiation, primarily through activation of the NF-kB pathway. The NF-kB pathway promotes lymphocyte proliferation and cell resistance to apoptosis (Bangham and Ratner, 2015). Tax also interacts with a plethora of other proteins and pathways, including NFAT, AP-1, TRAF6, cell cycle inhibitors, cyclins, cyclin-dependent kinases, and PDZ-containing proteins, to promote further genetic instability and oncogenesis (Choi and Harhaj, 2014).

Once tumor formation occurs, Tax protein expression is repressed while HBZ continues to be ubiquitously expressed in ATL cells and HTLV-1 cells. HBZ promotes the expression of hot genes telomerase reverse transcriptase (hTERT) and JunD, which mitigates the effect of repeated mitosis on cellular senescence, thus promoting the survival of the tumor. Tax and HBZ have opposite effects on several pathways. Tax activates, and HBZ inhibits CREB, AP-1, NF-kB,

telomerase, and Wnt; HBZ activates and Tax inhibits TGF-β. Tax and HBZ coordinate functions in a sophisticated manner to promote oncogenesis (Mulherkar *et al*., 2022).

In addition, viral infections may impair mounting an effective immune response to a subsequent bacterial infection alone or mixed infections where the host pathological levels of sepsis and immune cell infiltration in the infections was much greater than with alone infection with viral or bacterial agents (Pelkonen *et al*., 2012).

The new advances in the development of molecular methods have improved our insights into the conclusive knowledge of the genetic characteristics of the *HTLV-1* genome as well as propagated our understanding of its pathogenesis and through the light to open the avenues of their increased roles and/or involvement in more severe clinical infections along with an increase in the related issues in the further years.

5.2.3. Typing of brain tumors and HTLV-1 infections.

Viral infection of oligodendrocytes generally results in cytolysis, either as a direct result of viral infection or as a consequence of inflammatory and immune responses. Demyelination and paralysis are common distinguishing features of these infections, particularly in the case of infection with MHV and TMEV (Banerjee *et al*., 2007).

The current study showed positive HTLV-1 qRT-PCR detection results in patients with various forms of brain tumors, were 10%, 6%, 18%, 4%, and 4% of Pilocytic Astrocytoma; Meningotheliomatous Meningioma; Glioblastoma Multiformi; Diffuse Fibrillary Astrocytoma and Transitional Meningioma, respectively, showed positive qRT-PCR results for HTLV-1 detection. Similarly

logical seems the finding of reactivities to papillomavirus 16 exclusively in GBM patients which confirms a previous report of association of this virus with glioblastoma (Vidone *et al.,* 2014; Ali *et al.,* 2019).

In contrast, HTLV-1 infection has previously been detected in astrocytes and in infiltrating lymphocytes from the lesions of HAM/TSP patients (Lehky, *et al.*, 1995, Moritoyo, et al., 1996). Also, Astrocytes have previously been shown to support infection by mouse hepatitis virus (MHV), HIV-1, Theiler's murine encephalomyelitis virus (TMEV), and HTLV-1 (Araujo and Hall, 2004).

Banerjee et al., (2007) speculate that CNS resident cells such as astrocytes and oligodendrocytes may be infected in vivo and that Tax1-induced cytokine gene expression further mediates neurotoxicity.

Astrocytes interact with endothelial cells to form the BBB and may potentially also function as antigen-presenting cells (Mendez *et al*., 1997).

Banerjee et al., (2007) showed that HTLV-1 infection of astrocytes leads to a much more vigorous induction of proinflammatory cytokine gene expression pattern compared to HTLV-2. Oligodendrocytes have been shown to be cellular targets for neurotropic viruses, including JC virus, MHV, TMEV, and herpes simplex virus type 1 (Parra *et al*.,1999; Fazakerley *et al*.,2003).

Dysregulation of these proinflammatory cytokines may ultimately contribute to increased transmigration of HTLV-1-infected lymphocytes into the CNS, destruction of astrocytes and oligodendrocytes, and manifestation of HAM/TSP. Genetically similar to HTLV-1, the association of HTLV-2 infection with neurodegenerative diseases is rare and controversial (Araujo *et al*.,2004).

Although, Caprariello *et al.,* (2012) are in the process of determining whether Tax1 sensitizes oligodendrocytes to apoptosis, that synergy between induction of proinflammatory cytokines and apoptosis may result either in direct or in bystander damage to both astrocytes and oligodendrocytes within the CNS of HAM/TSP patients.

5.3. Molecular Study

The overall distribution of cytokines genotype and allele studied in benign and malignant brain tumor cases and controls. Cytokines production varies among individuals, due in part to genetic factors and, in particular, the presence of polymorphisms in important regulatory regions, such as promoter regions.

5.3.1. TNF-α polymorphism in patients with brain tumors

Cytokines are proposed to play important roles in brain tumor biology as well as neurodegeneration or impaired neuronal function. Cytokine gene polymorphisms showed a pattern of association with brain tumors, which may have a potential impact on family counseling and disease management (Settin *et al*., 2008).

The current results of TNF- α showed that DNA polymorphism distribution was according to GG; GA and AA were 40%, 30%, and 30%, respectively, in patients with brain tumors and 80%; 4% and 16%, respectively, in control group. The difference in the frequency of genotype distribution of the polymorphism between patients and control groups was statistically significant. This implies that patients carrying allele G of this polymorphism have about a 1.3-time chance of developing cancer compared with those carrying allele A, regardless of other characteristics.

Settin *et al*. (2008) found that TNF-α-308 A/A was more likely to be detected in nonmalignant and malignant brain cases than in controls. TNF-α-308 G/A was found to be significantly lower between both groups than in controls. Significant frequency higher TNF-α-308 A/A.

Waters *et al*. (2013) TNF-α-308 was identified as having a likely association. The TNF-α-308 SNP was further evaluated, and a significant association was identified, with 39% of allele 2 carriers having an unfavorable outcome compared with 31% of non-carriers (adjusted odds ratio 1.67, confidence interval 1.19–2.35, p=0.003).

Kamdee *et al*., (2021) indicated that subjects carrying the TNF-α-308 GA and AA genotypes and the A allele had a higher risk of IS development. In line with these findings, previous reports have shown that the TNF-α-308 A allele was associated with a risk of cerebral infarction in Korean populations (Um and Kim, 2004) and that North Indian subjects carrying TNF-α-308 GA and AA genotypes had a higher risk of coronary artery disease (Kumari *et al*.,2018). By contrast, the TNF-α-308G/A polymorphism was not associated with IS risk in the Chinese population (Gu *et al*. 2016) but was associated with protection against IS in East Asians (Tong *et al*. 2010; Song and Cheng, 2017).

Moreover, amongst healthy individuals, TNF-α-308 GA and AA genotypes had lower TNF-α levels compared with the GG genotype (Cui et al., 2012). Inconclusive cytokine expression results may depend on multiple non-genetic and genetic factors, as well as their interaction, leading to variability in the expression levels (Akhter *et al*., 2019). The interactions of other SNPs located in the inflammatory gene's promoter region or gene-gene interactions (Linderson, *et al*., 2005) may affect the overall transcriptional activity of gene promoters. However,

other SNPs located in the promoter region of the IL-6 and TNF-α gene and the interactions of each SNP are yet to be verified.

Infiltrating macrophages represented a significant population of non-neoplastic cells within malignant gliomas, in which they were the exclusive producers of TNF. Macrophages alter brain tumor development through a TNF-dependent process that culminates in the formation of microcysts. Macrophages and some of their secreted products, especially TNF, act as tumor promoters, and inhibition of these inflammatory components are currently regarded as potential therapeutic tools to block tumor progression (Villeneuve *et al*., 2005).

5.3.2. IFN-γ polymorphism in patients with brain tumors

Interferon-gamma is a key factor in viral infections involved in several immunological pathways, such as antigen processing and presentation, apoptosis, antiviral mediators, lysosome-mediated killing/phagosome maturation, and complement pathway, among others (Kak *et al*., 2018).

The present results showed that DNA polymorphism distribution according to CC; CA and AA were 44%, 34%, and 22%, respectively, in patients with brain tumors and 72%; 12% and 16%, respectively in the control group. There are significant statistical differences ($p< 0.05$) between studied groups according to genotyping of IFN-γ. This implies that patients carrying allele C of this polymorphism have about a 1.4-time chance of developing cancer compared with those carrying allele A, regardless of other characteristics.

IFN-γ, an important proinflammatory cytokine, is commonly associated with the pathogenesis of HAM (Neco, *et al*., 2017). Among asymptomatic individuals, the polymorphic allele for the IFN-γ +874A/T polymorphism was associated with higher levels of IFN-γ and proviral load. A more constant follow-up was suggested

for these patients to detect possible infection-related symptoms (Queiroz, *et al*., 2018).

The investigation of IFN-γ (+874T/A) gene polymorphisms among asymptomatic and symptomatic HTLV-1-infected subjects and in control individuals showed that IFN-γ +874A/T genotype was associated with a higher proviral load than +874AA genotype (Rocha-Junior *et al*., 2012).

By analogy, An *et al*., (2003) investigated the effects of single nucleotide polymorphisms (SNPs), -179G/T, in the promoter of the interferon-gamma gene (that confers differential TNF-alpha inducibility to the IFN-γ promoter). This study observed polymorphism's effects on the CD4+ T cell depletion rate in 298 African American HIV-1 seroconverts. The authors found that the IFN-γ -179G/T genotype was associated with an accelerated decline of CD4+ T cells to less than 200 cells/mm3 and a diagnosis of AIDS. This indicated that IFN-γ -179T is a risk factor for AIDS progression. Their results showed that when -179T (that is rarer than -179G) was specifically present, TNF-alpha induced elevated production of IFN-γ and a more rapid loss of CD4+ T cells. Therefore, certain genetic polymorphisms in the IFN-γ promoter favor greater production of IFN-γ.

IFN-gamma-associated macrophage stimulation results in the secretion of neopterin that is associated with the progression of CNS HIV-1 infection, and IFN-gamma-stimulated macrophages increase the production of quinolinic acid, an excitotoxin, and convulsant (Heyes *et al*., 1992).

Shapshak *et al*. (2004) results support the role of IFN-gamma in the pathogenesis of neuropsychiatric disease in HIV-1 infection. Attenuation of macrophage-related toxicity is central in the treatment of HIV-1-associated

inflammation in the brain, the pathological term for which is HIV encephalitis (HIVE).

5.3.3. KIR polymorphism in patients with brain tumors

An important HLA class I activity is its binding to killer immunoglobulin-like receptors (KIR) expressed on the surface of NK cells. The HLA-KIR interaction may promote the inhibition or the activation of NK cell functions (Sivori *et al*., 2019). Genes that code for KIR present a large genetic variability, resulting in different types of receptors (Nakimuli *et al*., 2015).

The results of the present study showed that DNA polymorphism distribution according to TT; AT and AA were 38%, 32%, and 30%, respectively, in patients with brain tumors and 76%; 8%, and 16%, respectively in the control group. Statistical comparison of these polymorphisms revealed significant differences. This implies that patients carrying allele T of this polymorphism have about a 1.3-time chance of developing cancer compared with those carrying allele A, regardless of other characteristics.

Gras Navarro *et al*. (2014) indicate that the KIR2DS2 gene may identify alloreactive NK cell subsets with higher potency against GBM, independently of the repertoire of inhibitory or other activating KIRs. KIR2DS4+ NK cells were more potent against GBM than KIR2DS2−/KIR2DS4− NK cells, but they were not significantly more potent than KIR2DS2+ NK cells.

In HTLV-1 infection, the KIR2DL2 gene can both enhance protection and impair HLA class I-mediated immunity against HTLV-1 infection. The activity of KIR2DL2 appears to enhance immunity against HTLV-1 through the activation of two cell types, NK cells and CD8+ T lymphocytes, both of which may exert better control in the development of clinical disease (Seich *et al*., 2011).

The evaluation of the protective effect of HLA-B*57 on different types of KIR (KIR2DL1+2+, KIR2DL2+C1+, KIR2DL2+C2+, KIR2DL3+C1+, KIR2DL1- or KIR2DL1+C2-, KIR2DL3- or KIR2DL3+C) showed that they all enhance HLA class I associations in HTLV-1 infection. These findings may be related to increased survival of CD8+ T cells in the presence of functional KIRs (Boelen *et al*., 2018).

Although the importance of HLA and KIR in the anti-viral immune response is recognized, there is a broader lack of studies involving different ethnic groups that seek to identify the possible impact of polymorphisms in these genes on HTLV-1 infection and associated diseases.

However, the KIR2DS2+ NK cells exhibited greater cytotoxic potency against all GBMs without HLA-A*11:01 ligands, as well as K562 cells lacking all HLA ligands. These findings might indicate that an intrinsic cytotoxic potency, rather than KIR–HLA ligand interactions, is the mechanism of potency associated with the KIR2DS2 immunogenotype. However, the existence of other unidentified ligands cannot be ruled out. They were unable to unequivocally demonstrate the impact of KIR2DS2 and KIR2DS4 because KIR2DS2 and KIR2DS4 receptors were expressed at the protein level on only a minor subpopulation of NK cells in contrast to NKG2D, which is constitutively expressed on all NK cells; the anti-KIR2DS2 and anti-KIR2DS4 Abs that they used are not known to have a blocking, neutralizing, or stimulating effect; and high linkage disequilibrium between KIR2DS2 and KIR2DL2 genes—only 4 aa residues distinguish them at the ligand-binding domain–means that Abs against KIR2DS2 might also recognize KIR2DL2. Generating highly specific neutralizing monoclonal anti-KIR2DS2 and anti-KIR2DS4 Abs, as well as an NK cell culture protocol that allows the selective expansion of KIR2DS2+ or KIR2DS4+ cells, might enable functional

investigations of the role of KIR2DS2 and KIR2DS4 receptors in NK cell–mediated lysis of tumor cells. Despite these shortcomings, they demonstrated functionally that KIR2DS2+-enriched NK cell subsets retained significantly higher levels of CD69 and CD16 and secreted significantly more sCD137, which might regulate their activation. The increased cytotoxic potential of KIR2DS2+ NK cells was consistent with significantly higher intrinsic levels of CD107a+ cytolytic lysosomes and increased granzyme A degranulation when in contact with GBM cells compared with KIR2DS2− NK cell subsets and NK cells from immunogenotype KIR2DS2. Human granzyme A is a serine protease with trypsin-like activity; it cleaves basic arginine and lysine residues to induce rapid caspase-independent cell death through ssDNA breaks (Lieberman and Fan, 2003).

Furthermore, they investigated the impact of KIR repertoires on the NK cells' potency against solid GBM in vivo and demonstrated that donor 3's NK cells, which possessed the KIR2DS2+ gene, prolonged median survival by 72 d compared with control and by 46 d compared with donor 4's KIR2DS2− NK cells, and manifested as decreased tumor proliferation, reduced angiogenesis, and increased apoptosis (Rygh *et al*., 2014).

5.3.4. IL1Ra polymorphism in patients with brain tumors:

Increased glial activation, pro-inflammatory cytokine concentration, BBB permeability, and leukocyte invasion are common events following brain injury and have been documented in neurodegenerative diseases; IL-1 receptor signaling also potently provokes the production of secondary inflammatory cytokines and chemokines such as IL-6, TNFα, and G-CSF, which ultimately indicate the central role of IL-1 in sterile inflammation (Retinasamy and Shaikh, 2023).

The presented results of rs2234679 showed that DNA polymorphism distribution was according to CC; CG and GG were 40%; 30% and 30%, respectively, in patients with brain tumors and 68%, 12%, and 20%, respectively in the control group. While the results of rs16065 showed that DNA polymorphism distribution were DNA polymorphism distributions according to CC; CT and TT were 54%, 16%, and 30%, respectively, in patients with brain tumors and 60%; 12% and 28%, respectively in the control group. The difference in frequency of genotype distribution of the polymorphism between patients and control groups was statistically non-significant.

On the other hand, Settin *et al*. (2008) revealed that these cases have shown no significant difference regarding the distribution of IL-1Ra VNTR genotype and allele polymorphism compared to controls.

In addition, previous studies showed no significant difference regarding the distribution of IL-1Ra VNTR genotype polymorphism compared to the control (Cvetkovic *et al*., 2005; El-Din, *et al*., 2009).

Hadjigeorgiou *et al*. (2005) compared with noncarriers IL-1RN allele 2 carriers had higher odds of having cerebral hemorrhages after TBI (adjusted OR = 4.57; 95% CI = 1.67 to 12.96; p = 0.004). The associations for (-511) IL-1B polymorphism were not significant.

Increased release of key pro-inflammatory factors and some cytokines, such as IL-1ß, TNFα, and interferon (IFN)-γ, are implicated in both the regulation of inflammation and the development of cancer. In fact, inflammatory cells and cytokines found in tumors can contribute to tumor growth, progression, and immunosuppression, as well as mounting an effective host anti-tumor response (Kilpinen *et al*. 2001).

Cytokines serve as one of the critical mediators of the inflammatory response, and one of the most extensively studied cytokines is the pro-inflammatory cytokine IL-1 (interleukin-1), which has been implicated in various chronic neurodegenerative disorders. The IL-1 family holds an additional member termed IL-1 receptor antagonist (IL-1Ra), which is encoded by the IL1RNgene. It serves as the single example of a naturally occurring antagonist molecule that competes with both IL-1α and IL-1β, thus decreasing inflammatory signaling and inhibiting them from further prompting the expression of other pro-inflammatory molecules (Chakrabarti et al., 2021). Based on the ability of IL-1Ra to inhibit neuronal loss could be an invaluable effective therapeutic agent for neurodegenerative diseases.

Our study's strength is that the first research investigating the possible association between IL-1RA VNTR polymorphism and the risk of brain tumors in the Iraqi population, though IL-1RA VNTR polymorphism has been studied as a potential susceptible marker before in other diseases. Secondly, our findings were more beneficial since patients with brain tumors and healthy participants were gathered from the same geographic area.

The first limitation was the small sample size, limiting the statistical power. Secondly, examination of additional clusters of genes and polymorphic sites related to IL-1RA would be needed to establish the contribution of IL-1RA VNTR gene polymorphisms to the etiology of brain tumors.

5.3.5. IL-28 polymorphism in patients with brain tumors:

IL-28 plays an important role in immune defense against viruses by inducing an "antiviral state" by intracellular signaling and activation of antiviral host factors in susceptible cells. The role of IL-28 in the context of infectious diseases may be

influenced mainly by polymorphisms in the gene that encodes the cytokine (Syedbasha and Egli, 2017).

The current results showed that DNA polymorphism distribution was according to GG; GC and CC were 46%; 20% and 34%, respectively in patients with brain tumors and 64%; 20%, and 16%, respectively in the control group. The statistical analysis of ***IFNL2*** **(or *IL-28A*)** polymorphism showed non-significant differences ($p>0.05$) between patients with brain tumor groups and control groups.

IL-28, or interferon lambda, is a type III interferon, an essential immune mediator that has been shown to have both antiviral as well as anti-tumor activity (Boisvert and Shoukry, 2016).

By analogy, Interferon-λ is among many secreted host proteins that activate an antiviral response. In previous work, Lazear *et al*. (2015a) observed that mice with genetic defects in interferon-λ signaling sustained greater West Nile virus infection in the brain and spinal cord, even though interferon-λ did not inhibit viral replication directly. Instead, interferon-λ signaling tightened the blood-brain barrier and limited West Nile virus dissemination into the brain. Administration of exogenous interferon-λ protected mice from West Nile virus infection in the brain and subsequent death. Thus, interferon-λ contributes to maintaining tissue barriers that restrict viral pathogenesis.

The brain is a partially immune-privileged site and is protected from most virus infections by effective peripheral clearance and multilayer physical barriers. Unfortunately, some neurotropic viruses, including α- and β-herpesviruses, represented by HSV-1 and HCMV, can still breach the defense and infect neurons and astrocytes. Using 5azadC and MS-275, which epigenetically rewire the IFNLR1 expression program in primary astrocytes, they demonstrated that the type

III IFN response could be remodeled to protect against both DNA and RNA viruses. Notably, MS-275 is capable of penetrating the blood–brain barrier (Simonini *et al*., 2006) and therefore has the potential to be used clinically in this respect.

Cancer has traditionally been viewed as a disease driven by the accumulation of genetic mutations. Lately, important roles for epigenetic abnormalities in cancer have come to light (Baylin *et al*., 2011).

Furthermore, the combination is also superior to IFN-λ alone due to its effectiveness in cancers previously insensitive to IFN-λ, including glioblastoma, which is known for its complexity and ability to develop resistance to single-agent therapy (Minniti *et al*., 2009).

A central question to IFN-λ regulation is how the restricted distribution of its receptor is achieved. Here, they dissected the role of epigenetic modifications and TFs in determining the tissue-specificity of IFN-λ receptor expression. They identified HDAC-mediated closed chromatin conformation as the major silencing mechanism of IFNLR1 expression in IFN-λ-insensitive cells (Ding, *et al.*, 2014).

By analogy, Only IL28B TT was significantly associated with HCC development after DAA treatment. IL28B genotype has long been evaluated as a factor conditioning response to interferon-based treatments, with genotype CC associated with more favorable results in terms of SVR (Chen *et al*., 2012; Hayes *et al*., 2012). When DAAs became available, this genotype was no longer a limiting factor since the potency of the new drugs overcame this factor of non-response (Gutierrez *et al*., 2015).

The antitumor activity is thought to imply the same mechanisms elucidated for type I interferons, inducing cell apoptosis and cell cycle arrest (Li *et al*., 2013). It

has been previously shown that the IL28B CT/TT polymorphisms were associated with progression to cirrhosis and HCC in viral hepatitis C, suggesting that the presence of these alleles represents a risk factor for HCC development coupled to cirrhosis per se (Fabris *et al*., 2011).

It is, therefore, tempting to speculate that in cirrhotic individuals carrying the unfavorable IL28 genotypes, this higher susceptibility to tumor development might be maintained after viral eradication because of a less effective immune response. IL28B codes for a member of the interferon lambda family; these molecules have essential functions in several biological events. They degrade viral genetic material and block viral replication showing antiviral effects. Besides the antiviral properties, interferon lambda shows other antitumor activities such as cell cycle arrest, apoptosis induction, enhancement of Natural Killer activity, and up-regulation of major histocompatibility complex, class I (MHC-I) (Donnelly *et al*.,2010; Laidlaw and Dustin, 2014).

Conclusions

And Recommendations

Conclusions

The following conclusions are obtained from the present study:

1- HTLV-1 is one of the most recently identified viruses in Iraqi patients with brain tumors as well as in the Iraqi population, and the present positive results of HTLV-1 in tissue samples from the patients group, might a proposed role for HTLV-1 at least as a cofactor in patients with brain tumors.

2- Age factor has a significant association with HTLV-1 in patients with brain tumors.

3- The current study indicated that TNF-alpha (TNF-α), Interferon-gamma (IFN-γ), and Killer Immunoglobulin-like Receptors (KIRs) genes polymorphism might be associated with risk factors in Iraqi patients for brain tumors. However, the exact roles and effects of parameters in brain tumors still need to be explored.

4- Serum levels of TNF-alpha (TNF-α); Interferon-gamma (IFN-γ); Killer Immunoglobulin-like Receptors (KIRs) as well as IL-1Ra can be considered useful diagnostic factors in brain tumors patients, like TNF- alpha(TNF-α); Interferon-gamma (IFN-γ); Killer Immunoglobulin-like Receptors (KIRs) genes polymorphisms.

5- In contrast: this study reached to a conclusion that indicated Interleukin 1 R a (IL-1Ra) and IL-28 genes polymorphism might be not-associated with a significant rate in this group of Iraqi patients with brain tumors. Yet, the exact role and effects of Interleukin 1 R a (IL-1Ra) and IL-28 gene

polymorphism in brain tumors need to be further studied to have clear views of their role in such tumors.

6- This study suggests that IL-1Ra might has a protective factor in this group of Iraqi patients with brain tumors.

7- Serum Interleukin -28 (IL-28) concentration level cannot be useful as diagnostic factors in patients with brain tumors, like Interleukin 1 R a (IL-1Ra) and IL-28 genes polymorphisms.

8- The significant statistical correlations between the gene polymorphism of TNF- alpha (TNF-α); Interferon-gamma (IFN-γ); Killer Immunoglobulin-like Receptors (KIRs); IL-1Ra with HTLV-1 infection could point to interactive roles between these molecular factors in brain tumorigenesis.

Recommendations

From the current results, it could be recommended the following:

1- HTLV-1 could be added as another potential cause of brain tumors in Iraqi populations.

2- Routine work uses viral detection such as (HHV-6, HHV-7, HSV1 and 2 …etc.) from blood as well as tissues, especially in young age, to prevent pathogenesis, inflammation, or carcinogenesis.

3- Further prospective studies required with a large number of cases are needed to validate the results of the current study, which may lead to a better understanding of the role of HTLV-1 in patients with brain tumors.

4- The exact role and effects of TNF-alpha (TNF-α), Interferon-gamma (IFN-γ), and Killer Immunoglobulin-like Receptors (KIRs) polymorphism in brain tumors are not fully identified, and therefore, for a better understanding of the association of their polymorphisms with brain tumors, we recommended further studies on different races and geographic areas with larger sample sizes to identify their effects on brain tumors.

5- Studying the role of other Genetic biomarkers in deregulating cell cycle pathways that play an essential role in brain tumors.

6- The use of highly sensitive RT-PCR test besides ELISA technique are important to confirm the early detection of HTLV-1 infection.

7- To study the complete whole genome sequence as well as the strains of HTLV-1 prevalent in Iraq and their molecular differences from the global one, attempting for preparation of vaccine that fits the Iraqi isolates is important.

8- Considering the role of cytokines in the signaling and activation of mediators of the innate and adaptive immune response, it seems important to broaden the analysis of SNPs in genes of pro and anti-inflammatory cytokines in the search to identify possible haplotypes associated with the regulatory and activating functions of the immune response.

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Appendices

Appendix (1): Recording of samples in NCBI, and the accession number of nucleotide sequences of TNF-α gene.

```
Homo sapiens tumor necrosis factor-alpha gene, partial cds.
PopSet: 2484506066

PopSet FASTA

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VERSION     OP380425.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
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REFERENCE   1  (bases 1 to 528)
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  TITLE     Direct Submission
  JOURNAL   Submitted (06-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
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  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (06-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
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            cds.
ACCESSION   OP380427
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VERSION     OP380427.1
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SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 528)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (06-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
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DEFINITION  Homo sapiens clone S4 tumor necrosis factor-alpha gene, partial
            cds.
ACCESSION   OP380428
VERSION     OP380428.1
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SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
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            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
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            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 528)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (06-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
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      181 aaacaaccct cagacgccac atcccctgac aagctgccag gcaggttctc ttcctctcac
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      301 tgatccggga cgtggagctg gccgaggagg cgctccccaa gaagacaggg gggccccagg
      361 gctccaggcg gtgcttgttc ctcagcctct tctccttcct gatcgtggca ggcgccacca
      421 cgctcttctg cctgctgcac tttggagtga tcggcccccа gagggaagag gtgagtgcct
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LOCUS       OP380429                 528 bp    DNA     linear   PRI 18-APR-2023
DEFINITION  Homo sapiens clone S5 tumor necrosis factor-alpha gene, partial
            cds.
ACCESSION   OP380429
VERSION     OP380429.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 528)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (06-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
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LOCUS       OP380430                 528 bp    DNA     linear   PRI 18-APR-2023
DEFINITION  Homo sapiens clone S6 tumor necrosis factor-alpha gene, partial
            cds.
ACCESSION   OP380430
VERSION     OP380430.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 528)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (06-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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FEATURES             Location/Qualifiers
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      301 tgatccggga cgtggagctg gccgaggagg cgctccccaa gaagacaggg gggccccagg
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LOCUS       OP380431                 528 bp    DNA     linear   PRI 18-APR-2023
DEFINITION  Homo sapiens clone S7 tumor necrosis factor-alpha gene, partial
            cds.
ACCESSION   OP380431
VERSION     OP380431.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 528)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (06-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
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      301 tgatccggga cgtggagctg gccgaggagg cgctccccaa gaagacaggg gggccccagg
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      421 cgctcttctg cctgctgcac tttggagtga tcggcccccа gagggaagag gtgagtgcct
      481 ggccagcctt catccactct cccacccaag gggaaatgga gacgcaag
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LOCUS       OP380432                 528 bp    DNA     linear   PRI 18-APR-2023
DEFINITION  Homo sapiens clone S8 tumor necrosis factor-alpha gene, partial
            cds.
ACCESSION   OP380432
VERSION     OP380432.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 528)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (06-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
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            ##Assembly-Data-END##
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      421 cgctcttctg cctgctgcac tttggagtga tcggcccccа gagggaagag gtgagtgcct
      481 ggccagcctt catccactct cccacccaag gggaaatgga gacgcaag
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Appendix (2): Recording of samples in NCBI, and the accession number of nucleotide sequences of IFN-γ gene.

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LOCUS       OP326723                 522 bp    DNA     linear   PRI 05-APR-2023
DEFINITION  Homo sapiens isolate S1 interferon-gamma (INFG) gene, partial cds.
ACCESSION   OP326723
VERSION     OP326723.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-AUG-2022) Clinical Laboratory analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla, Hilla, Iraq 51001, Iraq
COMMENT     ##Assembly-Data-START##
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      301 aacctaagca agatcccatg ggttgtgtgt ttatttcact tgatgataca atgaacactt
      361 ataagtgaag tgatactatc cagttactgc cggtttgaaa atatgcctgc aatctgagcc
      421 agtgctttaa tggcatgtca gacagaactt gaatgtgtca ggtgaccctg atgaaaacat
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LOCUS       OP326724                 522 bp    DNA     linear   PRI 05-APR-2023
DEFINITION  Homo sapiens isolate S2 interferon-gamma (INFG) gene, partial cds.
ACCESSION   OP326724
VERSION     OP326724.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-AUG-2022) Clinical Laboratory analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla, Hilla, Iraq 51001, Iraq
COMMENT     ##Assembly-Data-START##
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LOCUS       OP326725                 522 bp    DNA     linear   PRI 05-APR-2023
DEFINITION  Homo sapiens isolate S3 interferon-gamma (INFG) gene, partial cds.
ACCESSION   OP326725
VERSION     OP326725.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-AUG-2022) Clinical Laboratory analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla, Hilla, Iraq 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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                     /country="Iraq"
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      361 ataagtgaag tgatactatc cagttactgc cggtttgaaa atatgcctgc aatctgagcc
      421 agtgctttaa tggcatgtca gacagaactt gaatgtgtca ggtgaccctg atgaaaacat
      481 agcatctcag gagatttcat gcctggtgct tccaaatatt gt
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LOCUS       OP326726                  522 bp    DNA     linear   PRI 05-APR-2023
DEFINITION  Homo sapiens isolate S4 interferon-gamma (INFG) gene, partial cds.
ACCESSION   OP326726
VERSION     OP326726.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-AUG-2022) Clinical Laboratory analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla, Hilla, Iraq 51001, Iraq
COMMENT     ##Assembly-Data-START##
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     CDS             <1..29
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                     /translation="FRGRRASQ"
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      181 tatatgtatt atttataatt cctatatcct gtgactgtct cacttaatcc tttgttttct
      241 gactaattag gcaaggctat gtgattacaa ggctttatct caggggccaa ctaggcagcc
      301 aacctaagca agatcccatg ggttgtgtgt ttatttcact tgatgataca atgaacactt
      361 ataagtgaag tgatactatc cagttactgc cggtttgaaa atatgcctgc aatctgagcc
      421 agtgctttaa tggcatgtca gacagaactt gaatgtgtca ggtgaccctg atgaaaacat
      481 agcatctcag gagatttcat gcctggtgct tccaaatatt gt
//
LOCUS       OP326727                 522 bp    DNA     linear   PRI 05-APR-2023
DEFINITION  Homo sapiens isolate S5 interferon-gamma (INFG) gene, partial cds.
ACCESSION   OP326727
VERSION     OP326727.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-AUG-2022) Clinical Laboratory analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla, Hilla, Iraq 51001, Iraq
COMMENT     ##Assembly-Data-START##
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      241 gactaattag gcaaggctat gtgattacaa ggctttatct caggggccaa ctaggcagcc
      301 aacctaagca agatcccatg ggttgtgtgt ttatttcact tgatgataca atgaacactt
      361 ataagtgaag tgatactatc cagttactgc cggtttgaaa atatgcctgc aatctgagcc
      421 agtgctttaa tggcatgtca gacagaactt gaatgtgtca ggtgaccctg atgaaaacat
      481 agcatctcag gagatttcat gcctggtgct tccaaatatt gt
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LOCUS       OP326728                  522 bp    DNA     linear   PRI 05-APR-2023
DEFINITION  Homo sapiens isolate S6 interferon-gamma (INFG) gene, partial cds.
ACCESSION   OP326728
VERSION     OP326728.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-AUG-2022) Clinical Laboratory analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla, Hilla, Iraq 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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                     /country="Iraq"
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                     /gene="INFG"
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      121 atcaatcaaa taagtattta taatagcaac ttttgtgtaa tgaaaatgaa tatctattaa
      181 tatatgtatt atttataatt cctatatcat gtgactgtct cacttaatcc tttgttttct
      241 gactaattag gcaaggctat gtgattacaa ggctttatct caggggccaa ctaggcagcc
      301 aacctaagca agatcccatg ggttgtgtgt ttatttcact tgatgataca atgaacactt
      361 ataagtgaag tgatactatc cagttactgc cggtttgaaa atatgcctgc aatctgagcc
      421 agtgctttaa tggcatgtca gacagaactt gaatgtgtca ggtgaccctg atgaaaacat
      481 agcatctcag gagatttcat gcctggtgct tccaaatatt gt
//
LOCUS       OP326729                 522 bp    DNA     linear   PRI 05-APR-2023
DEFINITION  Homo sapiens isolate S7 interferon-gamma (INFG) gene, partial cds.
ACCESSION   OP326729
VERSION     OP326729.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-AUG-2022) Clinical Laboratory analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla, Hilla, Iraq 51001, Iraq
COMMENT     ##Assembly-Data-START##
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     CDS             <1..29
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                     /translation="FRGRRASQ"
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      241 gactaattag gcaaggctat gtgattacaa ggctttatct caggggccaa ctaggcagcc
      301 aacctaagca agatcccatg ggttgtgtgt ttatttcact tgatgataca atgaacactt
      361 ataagtgaag tgatactatc cagttactgc cggtttgaaa atatgcctgc aatctgagcc
      421 agtgctttaa tggcatgtca gacagaactt gaatgtgtca ggtgaccctg atgaaaacat
      481 agcatctcag gagatttcat gcctggtgct tccaaatatt gt
//
LOCUS       OP326730                 522 bp    DNA     linear   PRI 05-APR-2023
DEFINITION  Homo sapiens isolate S8 interferon-gamma (INFG) gene, partial cds.
ACCESSION   OP326730
VERSION     OP326730.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-AUG-2022) Clinical Laboratory analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla, Hilla, Iraq 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /country="Iraq"
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     mRNA            <1..>522
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      121 atcaatcaaa taagtattta taatagcaac ttttgtgtaa tgaaaatgaa tatctattaa
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      241 gactaattag gcaaggctat gtgattacaa ggctttatct caggggccaa ctaggcagcc
      301 aacctaagca agatcccatg ggttgtgtgt ttatttcact tgatgataca atgaacactt
      361 ataagtgaag tgatactatc cagttactgc cggtttgaaa atatgcctgc aatctgagcc
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LOCUS       OP326731                 522 bp    DNA     linear   PRI 05-APR-2023
DEFINITION  Homo sapiens isolate S9 interferon-gamma (INFG) gene, partial cds.
ACCESSION   OP326731
VERSION     OP326731.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 522)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-AUG-2022) Clinical Laboratory analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla, Hilla, Iraq 51001, Iraq
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      121 atcaatcaaa taagtattta taatagcaac ttttgtgtaa tgaaaatgaa tatctattaa
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      241 gactaattag gcaaggctat gtgattacaa ggctttatct caggggccaa ctaggcagcc
      301 aacctaagca agatcccatg ggttgtgtgt ttatttcact tgatgataca atgaacactt
      361 ataagtgaag tgatactatc cagttactgc cggtttgaaa atatgcctgc aatctgagcc
      421 agtgctttaa tggcatgtca gacagaactt gaatgtgtca ggtgaccctg atgaaaacat
      481 agcatctcag gagatttcat gcctggtgct tccaaatatt gt
//
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Appendix (3): Recording of samples in NCBI, and the accession number of nucleotide sequences of KIR gene.

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LOCUS       OP373670                 530 bp    DNA     linear   PRI 12-APR-2023
DEFINITION  Homo sapiens isolate S1 KIR2DS2 killer-cell immunoglobulin-like
            receptor (KIR2DS2) gene, partial cds.
ACCESSION   OP373670
VERSION     OP373670.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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      241 cctcattgga gagcaccatg atggggtctc caaggccaac ttctccatcg gtcccatgat
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      361 gtcagctccc agtgaccctc tggacatcgt catcacaggt gagagtgtcc ggacattctc
      421 attgtcattg ggctgcagag tgaatgatcc acgacttgga acccccaggt agttgtaagg
      481 aagatgagct tggtattctt atggagagag actgacttgc tgaggtttgt
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LOCUS       OP373671                 530 bp    DNA     linear   PRI 12-APR-2023
DEFINITION  Homo sapiens isolate S2 KIR2DS2 killer-cell immunoglobulin-like
            receptor (KIR2DS2) gene, partial cds.
ACCESSION   OP373671
VERSION     OP373671.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /chromosome="19"
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DEFINITION  Homo sapiens isolate S3 KIR2DS2 killer-cell immunoglobulin-like
            receptor (KIR2DS2) gene, partial cds.
ACCESSION   OP373672
VERSION     OP373672.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
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LOCUS       OP373673                 530 bp    DNA     linear   PRI 12-APR-2023
DEFINITION  Homo sapiens isolate S4 KIR2DS2 killer-cell immunoglobulin-like
            receptor (KIR2DS2) gene, partial cds.
ACCESSION   OP373673
VERSION     OP373673.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
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LOCUS       OP373674                 530 bp    DNA     linear   PRI 12-APR-2023
DEFINITION  Homo sapiens isolate S5 KIR2DS2 killer-cell immunoglobulin-like
            receptor (KIR2DS2) gene, partial cds.
ACCESSION   OP373674
VERSION     OP373674.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
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      301 gcaagacctt gcagggacct acagatgcta cggttctgtt actcactccc cctatcagtt
      361 gtcagctccc agtgaccctc tggacatcgt catcacaggt gagagtgtcc ggacattctc
      421 attgtcattg ggctgcagag tgaatgatcc acgacttgga acccccaggt agttgtaagg
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LOCUS       OP373675                 530 bp    DNA     linear   PRI 12-APR-2023
DEFINITION  Homo sapiens isolate S6 KIR2DS2 killer-cell immunoglobulin-like
            receptor (KIR2DS2) gene, partial cds.
ACCESSION   OP373675
VERSION     OP373675.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
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      421 attgtcattg ggctgcagag tgaatgatcc acgacttgga acccccaggt agttgtaagg
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LOCUS       OP373676                 530 bp    DNA     linear   PRI 12-APR-2023
DEFINITION  Homo sapiens isolate S7 KIR2DS2 killer-cell immunoglobulin-like
            receptor (KIR2DS2) gene, partial cds.
ACCESSION   OP373676
VERSION     OP373676.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
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LOCUS       OP373677                 530 bp    DNA     linear   PRI 12-APR-2023
DEFINITION  Homo sapiens isolate S8 KIR2DS2 killer-cell immunoglobulin-like
            receptor (KIR2DS2) gene, partial cds.
ACCESSION   OP373677
VERSION     OP373677.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 530)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 530)
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  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
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Appendix (4): Recording of samples in NCBI, and the accession number of nucleotide sequences of IL1Ra gene.

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LOCUS       OP351527                 477 bp    DNA     linear   PRI 11-APR-2023
DEFINITION  Homo sapiens isolate S1 interleukin 1 receptor antagonist (IL1RN)
            gene, partial cds.
ACCESSION   OP351527
VERSION     OP351527.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-AUG-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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LOCUS       OP351528                 477 bp    DNA     linear   PRI 11-APR-2023
DEFINITION  Homo sapiens isolate S2 interleukin 1 receptor antagonist (IL1RN)
            gene, partial cds.
ACCESSION   OP351528
VERSION     OP351528.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-AUG-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
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            ##Assembly-Data-END##
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      181 cctccttgta ctctctgagg tgctctggaa ggaggggcag ctccaccctg ggagggactg
```

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      241 tggcccaggt actgcccggg tgctacttta tgggcagcag ctcagttgag ttagagtctg
      301 gaagacctca gaagacctcc tgtcctatga ggccctcccc atggctttag gtaagctcct
      361 tccactctca ttttttcacc tgagaaatga gagaggaaaa tgtctacaat tggtgtttat
      421 caaatgcttt caggctctgg tgagcaagcg tccaggaaaa tgtcaagcgc atggagc
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LOCUS       OP351529                 477 bp    DNA     linear   PRI 11-APR-2023
DEFINITION  Homo sapiens isolate S3 interleukin 1 receptor antagonist (IL1RN)
            gene, partial cds.
ACCESSION   OP351529
VERSION     OP351529.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-AUG-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
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      241 tggcccaggt actgcccggg tgctacttta tgggcagcag ctcagttgag ttagagtctg
      301 gaagacctca gaagacctcc tgtcctatga ggccctcccc atggctttag gtaagctcct
      361 tccactctca ttttttcacc tgagaaatga gagaggaaaa tgtctacaat tggtgtttat
      421 caaatgcttt caggctctgg tgagcaagcg tccaggaaaa tgtcaagcgc atggagc
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LOCUS       OP351530                 477 bp    DNA     linear   PRI 11-APR-2023
DEFINITION  Homo sapiens isolate S4 interleukin 1 receptor antagonist (IL1RN)
            gene, partial cds.
ACCESSION   OP351530
VERSION     OP351530.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-AUG-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
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      181 cctccttgta ctctctgagg tgctctggaa ggaggggcag ctccaccctg ggagggactg
      241 tggcccaggt actgcccggg tgctacttta tgggcagcag ctcagttgag ttagagtctg
      301 gaagacctca gaagacctcc tgtcctatga ggccctcccc atggctttag gtaagctcct
      361 tccactctca ttttttcacc tgagaaatga gagaggaaaa tgtctacaat tggtgtttat
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LOCUS       OP351531                 477 bp    DNA     linear   PRI 11-APR-2023
DEFINITION  Homo sapiens isolate S5 interleukin 1 receptor antagonist (IL1RN)
            gene, partial cds.
ACCESSION   OP351531
VERSION     OP351531.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-AUG-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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                     /chromosome="2"
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                     /country="Iraq"
                     /collection_date="Nov-2021"
                     /collected_by="Ataa Khalil Al-Shamari"
     gene            <341..>477
                     /gene="IL1RN"
     mRNA            <341..>350
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                     /protein_id="WFG82991.1"
                     /translation="MAL"
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       181 cctccttgta ctctctgagg tgctctggaa ggaggggcag ctccaccctg ggagggactg
       241 tggcccaggt actgcccggg tgctacttta tgggcagcag ctcagttgag ttagagtctg
       301 gaagacctca gaagacctcc tgtcctatga ggccctcccc atggctttag gtaagctcct
       361 tccactctca ttttttcacc tgagaaatga gagaggaaaa tgtctacaat tggtgtttat
       421 caaatgcttt caggctctgg tgagcaagcg tccaggaaaa tgtcaagcgc atggagc
//
LOCUS       OP351532                 477 bp    DNA     linear   PRI 11-APR-2023
DEFINITION  Homo sapiens isolate S6 interleukin 1 receptor antagonist (IL1RN)
            gene, partial cds.
ACCESSION   OP351532
VERSION     OP351532.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-AUG-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /chromosome="2"
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                     /country="Iraq"
                     /collection_date="Nov-2021"
                     /collected_by="Ataa Khalil Al-Shamari"
     gene            <341..>477
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     mRNA            <341..>350
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                     /protein_id="WFG82992.1"
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ORIGIN
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      121 ccggcccatc tcctcatgct ggccaaccct ctgtgagtgt gtgggagggg aggctgggct
      181 cctccttgta ctctctgagg tgctctggaa ggaggggcag ctccaccctg ggagggactg
      241 tggcccaggt actgcccggg tgctacttta tgggcagcag ctcagttgag ttagagtctg
      301 gaagacctca gaagacctcc tgtcctatca ggccctcccc atggctttag gtaagctcct
      361 tccactctca ttttttcacc tgagaaatga gagaggaaaa tgtctacaat tggtgtttat
      421 caaatgcttt caggctctgg tgagcaagcg tccaggaaaa tgtcaagcgc atggagc
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LOCUS       OP351533                 477 bp    DNA     linear   PRI 11-APR-2023
DEFINITION  Homo sapiens isolate S7 interleukin 1 receptor antagonist (IL1RN)
            gene, partial cds.
ACCESSION   OP351533
VERSION     OP351533.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-AUG-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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                     /chromosome="2"
                     /tissue_type="brain tumors"
                     /country="Iraq"
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     mRNA            <341..>350
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      121 ccggcccatc tcctcatgct ggccaaccct ctgtgagtgt gtgggagggg aggctgggct
      181 cctccttgta ctctctgagg tgctctggaa ggaggggcag ctccaccctg ggagggactg
      241 tggcccaggt actgcccggg tgctacttta tgggcagcag ctcagttgag ttagagtctg
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      361 tccactctca ttttttcacc tgagaaatga gagaggaaaa tgtctacaat tggtgtttat
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LOCUS       OP351534                 477 bp    DNA     linear   PRI 11-APR-2023
DEFINITION  Homo sapiens isolate S8 interleukin 1 receptor antagonist (IL1RN)
            gene, partial cds.
ACCESSION   OP351534
VERSION     OP351534.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-AUG-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
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                     /chromosome="2"
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      181 cctccttgta ctctctgagg tgctctggaa ggaggggcag ctccaccctg ggagggactg
      241 tggcccaggt actgcccggg tgctacttta tgggcagcag ctcagttgag ttagagtctg
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      361 tccactctca ttttttcacc tgagaaatga gagaggaaaa tgtctacaat tggtgtttat
      421 caaatgcttt caggctctgg tgagcaagcg tccaggaaaa tgtcaagcgc atggagc
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LOCUS       OP351535                 477 bp    DNA     linear   PRI 11-APR-2023
DEFINITION  Homo sapiens isolate S9 interleukin 1 receptor antagonist (IL1RN)
            gene, partial cds.
ACCESSION   OP351535
VERSION     OP351535.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-AUG-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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      121 ccggcccatc tcctcatgct ggccaaccct ctgtgagtgt gtgggagggg aggctgggct
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LOCUS       OP351536                 477 bp    DNA     linear   PRI 11-APR-2023
DEFINITION  Homo sapiens isolate S10 interleukin 1 receptor antagonist (IL1RN)
            gene, partial cds.
ACCESSION   OP351536
VERSION     OP351536.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and Immunological Study of Human T- Lymphotropic Virus
            type I: (HTLV-I) in Patients with Brain Tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 477)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-AUG-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /chromosome="2"
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                     /country="Iraq"
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                     /collected_by="Ataa Khalil Al-Shamari"
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      361 tccactctca ttttttcacc tgagaaatga gagaggaaaa tgtctacaat tggtgtttat
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Appendix (5): Recording of samples in NCBI, and the accession number of nucleotide sequences of IL28 gene.

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LOCUS       OP359987                 604 bp    DNA     linear   PRI 16-APR-2023
DEFINITION  Homo sapiens isolate S1 interleukin 28A (IL28A) gene, partial cds.
ACCESSION   OP359987
VERSION     OP359987.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and immunological study of human t-lymphotropic virus
            type I: (HTLV-I) in patients with brain tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
COMMENT     ##Assembly-Data-START##
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            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and immunological study of human t-lymphotropic virus
            type I: (HTLV-I) in patients with brain tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
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            Sequencing Technology :: Sanger dideoxy sequencing
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DEFINITION  Homo sapiens isolate S3 interleukin 28A (IL28A) gene, partial cds.
ACCESSION   OP359989
VERSION     OP359989.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
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            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and immunological study of human t-lymphotropic virus
            type I: (HTLV-I) in patients with brain tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
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            Sequencing Technology :: Sanger dideoxy sequencing
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DEFINITION  Homo sapiens isolate S4 interleukin 28A (IL28A) gene, partial cds.
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VERSION     OP359990.1
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SOURCE      Homo sapiens (human)
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REFERENCE   1  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and immunological study of human t-lymphotropic virus
            type I: (HTLV-I) in patients with brain tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
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            Sequencing Technology :: Sanger dideoxy sequencing
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VERSION     OP359991.1
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REFERENCE   1  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and immunological study of human t-lymphotropic virus
            type I: (HTLV-I) in patients with brain tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
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            Sequencing Technology :: Sanger dideoxy sequencing
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ACCESSION   OP359992
VERSION     OP359992.1
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  ORGANISM  Homo sapiens
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            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and immunological study of human t-lymphotropic virus
            type I: (HTLV-I) in patients with brain tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
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LOCUS       OP359993                  604 bp    DNA     linear   PRI 16-APR-2023
DEFINITION  Homo sapiens isolate S7 interleukin 28A (IL28A) gene, partial cds.
ACCESSION   OP359993
VERSION     OP359993.1
KEYWORDS    .
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REFERENCE   1  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and immunological study of human t-lymphotropic virus
            type I: (HTLV-I) in patients with brain tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
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DEFINITION  Homo sapiens isolate S8 interleukin 28A (IL28A) gene, partial cds.
ACCESSION   OP359994
VERSION     OP359994.1
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SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
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            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and immunological study of human t-lymphotropic virus
            type I: (HTLV-I) in patients with brain tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
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  TITLE     Direct Submission
  JOURNAL   Submitted (01-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
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            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
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            type I: (HTLV-I) in patients with brain tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
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DEFINITION  Homo sapiens isolate S10 interleukin 28A (IL28A) gene, partial cds.
ACCESSION   OP359996
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SOURCE      Homo sapiens (human)
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REFERENCE   1  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Molecular and immunological study of human t-lymphotropic virus
            type I: (HTLV-I) in patients with brain tumors
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 604)
  AUTHORS   Al-Shamari,A.K. and Mohammed,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-SEP-2022) Clinical Laboratory Analysis, Al-Mustaqbal
            University College, Al-Tagia, Hilla 51001, Iraq
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الخلاصة

فايروس تي اللمفاوي البشري من النوع الأول هو العامل المسبب لسرطان الخلايا اللمفاوية / ابيضاض الدم لدى البالغين ولاضطرابات الجهاز العصبي المرتبطة بـالفيروس، بما في ذلك الخزل التشنجي المداري/ الاعتلال النقوي المرتبط بالفيروس. تلعب الحركيات الخلوية دورا مهما في حيوية الأورام الدماغية، التنكُّس العصبي، كما تسبب ضعف الوظيفة العصبية في الدماغ. وقد تؤثر التغايرات الوراثية الوظيفية في جينات عامل النخر الورمي-الفا ومضادات مستقبلات إنترلوكين ١ في حالات الإصابة بأمراض الدماغ. انترفيرون-كاما له دور في تنظيم استجابات الجهاز المناعي للمضيف. كما وتتميز مستقبلات الغلوبولين والانترلوكين-٢٨ بقدرتها على تحفيز المقاومة والاستجابة المناعية ضد العدوى الفيروسية.

تهدف هذه الدراسة إلى تحديد نسبة انتشار فايروس تي اللمفاوي البشري من النوع الأول ودراسة التغايرات الوراثية في عامل النخر الورمي ألفا، إنترفيرون كاما، مستقبلات القاتلة الشبيهة الغلوبيولين، مضاد مستقبلات الإنترلوكين ١، والإنترلوكين- ٢٨ ومدى تأثيرها ودورها في مرضى أورام الدماغ لعينات من المجتمع العراقي.

شملت الدراسة الحالية دراسة مستعرضة للحالات-السيطرة مئة وخمسين (١٥٠) عينة تضمنت، خمسة وسبعين (٧٥) عينة مختارة عشوائيا من الدم وأنسجة اورام الدماغ لأنواع مختلفة لمرضى تراوحت أعمارهم بين ٢ الى ٨٥ عامًا. بالإضافة الى ذلك تم جمع خمسة وعشرين (٢٥) عينة كمجموعة سيطرة من الجثث المشرحة والذين تراوحت اعمارهم بين ١٦ الى ٧١ عامًا والتي تم الحصول عليها من افراد متوفيين بأسباب غير عصبية، والتي تظهر أنسجة دماغ طبيعية تشريحيا. وكما تم جمع خمسون (٥٠) عينة دم من أشخاص أصحاء ظاهريًا كمجموعة سيطرة للمقارنة من المستشفيات التعليمية المختلفة في الفرات الاسط ومحافظة بغداد في العراق. حيث تم جمع عينات الدراسة الحالية خلال الفترة من كانون الثاني الى ايلول ٢٠٢٢.

جُمِعتُ أنسجة الدماغ، بالإضافة إلى عينات الدم بحجم ثلاثة إلى خمسة مللي لتر، حيث تم استخلاص الجينوم الفيروسي والحمض النووي الكلي وتخزينهما عند درجة حرارة -٧٠ درجة مئوية لحين الاستخدام. وتضمنت محاور الدراسة الكشف عن فايروس تي اللمفاوي البشري من النوع الأول باستخدام تقنية تفاعل البوليميراز المتسلسل اللحظي وإجراء فحوصات أخرى لتحديد تعدد الأشكال الجينية مثل جينات (عامل التنخر الورم-ألفا، إنترفيرون كاما، مستقبلات القاتلة الشبيهة الغلوبيولين، مضاد مستقبلات الإنترلوكين ١ والإنترلوكين- ٢٨) باستخدام تقنية سانجر للتسلسل الجيني . وكذلك تقدير التركيز المصلية لعامل التنخر الورم-ألفا، إنترفيرون

كاما، مستقبلات القاتلة الشبيهة الغلوبيولين، مضاد مستقبلات الإنترلوكين ١ والإنترلوكين- ٢٨ للمرضى الذين يعانون من أورام الدماغ ومجموعة السيطرة بواسطة المقايسة الامتصاصية المناعية للانزيم المرتبط.

ارتبطت عينات الأنسجة بالمرضى الذين يعانون من أورام في الدماغ بمتوسط عمر يبلغ ٥٠,٧ ± ١٠,٤ سنة، مقارنة بـ ٤٧,٩ ± ١٢,٦ سنة لمجموعة السيطرة. تتمثل اعداد الذكور ٤١ (٥٤,٧٪) من حالات أورام الدماغ، في حين بلغت اعداد الإناث ٣٤ (٤٥,٣٪). وان نسبة الذكور إلى الإناث كانت ١,٢: ١، بينما تألفت مجموعة السيطرة من ٤٥ ذكرا (٦٠٪) و٣٠ إناث (٤٠٪).

وجد ان الفئة الأولى من حالات اورام الدماغ كانت في ٣٧ حالة (٤٩,٣٪) موزعة (٢٠ ذكور و١٧ إناث)، الفئة الثانية في ١٧ حالة (٢٢,٧٪) (٩ ذكور و٨ إناث)، أما الفئة الرابعة فكانت في ١٣ حالة بنسبة (١٧,٣٪) (٧ ذكور و٦ إناث). في حين كانت الفئة الثالثة من الورم في ٨ حالات فقط (١٠,٧٪) في (٥ ذكور و٣ إناث).

احدى وعشرون ٢١ حالة من أورام الدماغ عبارة عن ورم نجمي شعري الخلايا (٢٨٪) (١١ ذكر و١٠ إناث)، ١٥ حالة من الورم القرابي السحائي (٢٠٪) (٨ ذكور و٧ إناث)، حالة ١٣ الورم الأرومي الدبقي متعدد الأشكال والورم النجمي الليفي المنتشر (١٧,٣٪) (٧ ذكور و٦ إناث)، ٦ حالات من ورم الدبقيات القليلة التغصن (٨٪) (٣ ذكور و٣ إناث)، ٤ حالات من الورم الغشائي الورم السحائي اللانمطي (٥,٤٪) (٣ ذكور و١ إنثى)، و٣ حالات من الورم الغشائي الانتقالي (٤٪) (٢ ذكور و١ إنثى)، توضح هذه البيانات اختلافات إحصائية كبيرة p=00.4 بين أورام الدماغ.

حُددت الإصابة الفيروسية في أورام الدماغ بنسبة ٥٠ (٦٦,٧%) من بين ٧٥ مريضًا بينما وجد ان ٢٥ (٣٣,٣%) مريضًا كانت نتيجة لأسباب اخرى. الفروق ذات دلالة إحصائية (p = 0.01) بين مجموعة المرضى. تظهر نتائج فحص تفاعل البوليميراز المتسلسل اللحظي ل فايروس تي اللمفاوي البشري من النوع الأول نسبة ٤٢% (٢١ من أصل ٥٠ حالة) إيجابية، بينما تظهر نسبة ٥٨% (٢٩ من أصل ٥٠ حالة) سلبية. كانت اعلى نسبة أنسجة أورام الدماغ المصابة ب فايروس تي اللمفاوي البشري من النوع الأول وفق الفئة العمرية (٧٠-٨٥ سنة)، والتي تمثل ٣٣,٣% (٧ من أصل ٢١حاله). ان نسبة أورام أنسجة الدماغ التي اعطت نتائج إيجابية لتقنية تفاعل البوليميراز المتسلسل اللحظي اعتمادا على جنس المرضى، حيث يمثل الذكور ٦١,٩% (١٣ من أصل ٢١ حالة) والإناث ٣٨,١% (٨ من أصل ٢١ حالة).

النتائج الموجبة للكشف عن فايروس الخلايا اللمفاوية البشري من النوع الأول باستخدام تقنية تفاعل البوليميراز المتسلسل اللحظي من المرضى الذين يعانون من أشكال مختلفة من أورام المخ هي ١٠٪ و٦٪

و١٨٪ و٤٪ و٤٪ من ورم نجمي شعري الخلايا ;ورم قرابي سحائي ;الورم الأرومي الدبقي ;ورم نجمي ليفي منتشر وورم سحائي انتقالي، على التوالي

أظهرت نتائج توزيع النمط الجيني ل عامل النخر الورمي الفا وحسب الأنماط الجينية GG، GAو AA كان ٤٠% و٣٠% و٣٠% على التوالي في مرضى أورام الدماغ و٨٠% و٤% و١٦% على التوالي في المجموعة السيطرة. كما تم توثيق تسجيلات جديدة في المركز الوطني لمعلومات التقانة الاحيائية وبنك الجينات تحت الرقم التسلسلي: BankIt2620154 OP380425، OP380426، OP380427، OP380428 ، OP380429،OP380430، OP380431، OP380432 .

نسبة توزيع الأنماط الجينية لجين انترفيرون -كاما CC، CA وAA كانت ٤٤% و٣٤% و٢٢% على التوالي في مرضى أورام الدماغ و٧٢% و١٢% و١٦% على التوالي في المجموعة السيطرة. كما تم توثيق تسجيلات جديدة في المركز الوطني لمعلومات التقانة الاحيائية وبنك الجينات تحت الرقم التسلسلي: BankIt 2617649 OP326723، OP326724، OP326725، OP326726، OP326727، OP326728، OP326729، OP326730، OP326731.

توزيع النمط الجيني لجين مستقبلات القاتلة الشبيهة الغلوبيولين وحسب الأنماط الجينية TT، AT، AA كانت ٣٨% ،٣٢% ،٣٠% على التوالي في مرضى أورام الدماغ ٧٦%، ٨%، ١٦% على التوالي في المجموعة السيطرة. تم توثيق تسجيلات جديدة في المركز الوطني لمعلومات التقانة الاحيائية وبنك الجينات تحت الرقم التسلسلي: OP373670 BankIt2619196، OP373671، OP373672، OP373673، OP373674، OP373675، OP373676، OP373677.

توزيع التنميط الجيني لجين مضاد مستقبلات الإنترلوكين ١ كان في(rs2234679) وفق الأنماط الجينية CC، CG، GG كان ٤٠%، ٣٠%، ٣٠% على التوالي، في المرضى الذين يعانون من أورام الدماغ، و٦٨%، ١٢%، ٢٠% على التوالي في مجموعة السيطرة. بينما كانت نتائج (rs16065) وفقًا للأنماط الجينية TT، TC، CC هي ٥٤%، ١٦%، ٣٠% على التوالي، في المرضى الذين يعانون من أورام الدماغ، و٦٠%، ١٢%، ٢٨% على التوالي في المجموعة السيطرة. أيضًا، تم توثيق تسجيلات جديدة في المركز الوطني لمعلومات التقانة الاحيائية وبنك الجينات تحت الرقم التسلسلي: BankIt2618097، OP351527، OP351528، OP351529، OP351530، OP351531، OP351532، OP351533، OP351534، OP351535، OP351536.

كما كانت ترددات التنميط الجيني لجين إنترلوكين- ٢٨وحسب الأنماط الجينية GG، GC، CC هي ٤٦%، ٢٠%، ٣٤% على التوالي، في المرضى الذين يعانون من أورام الدماغ، و٦٤%، ٢٠%، ١٦% على التوالي في المجموعة السيطرة. أيضًا، تم توثيق تسجيلات جديدة في المركز الوطني لمعلومات التقانة الاحيائية وبنك الجينات تحت الرقم التسلسلي: BankIt2618865، OP359987، OP359988، OP359989، OP359990، OP359991، OP359992، OP359993، OP359994، OP359995، OP359996.

إحصائيًا، وجدت فروق معنوية ($p<0.05$) عند مقارنة المستويات المصلية لعامل التنخر الورم-ألفا، إنترفيرون- كاما، مستقبلات القاتلة الشبيهة الغلوبيولين ومضاد مستقبلات الإنترلوكين- ١, بينما وجد اختلاف غير معنوي ($p<0.05$) في متوسط التركيز المصلي للإنترلوكين- ٢٨ بين مجاميع الدراسة.

ان استنتاجات الدراسة الحالية هي أن فايروس تي اللمفاوي البشري من النوع الأول يمكن أن يكون عامل مساعد في مسارات التكوين والأحداث الحيوية المتعلقة بأورام الدماغ لدى المرضى العراقيين، وقد تلعب الاختلافات الوراثية والتي تترافق مع زيادة تركيز عامل التنخر الورمي-ألفا وإنترفيرون- كاما ومستقبلات القاتلة الشبيهة الغلوبيولين، أدواراً مهمة كعوامل خطر آلية تكون أورام الدماغ المجهولة الأسباب. وبالمقابل، قد يلعب مضاد مستقبلات الإنترلوكين ١ دوراً وقائياً في المرضى العراقيين الذين يعانون من أورام دماغية. وأخيراً، فإن وجود الإنترلوكين- ٢٨ لا يمثل عاملاً تشخيصياً يمكن الاعتماد عليه في المرضى الذين يعانون من أورام الدماغ.

جمهـــــــــورية العـــــــــراق
وزارة التعليم والعالي والبحث العلمي
جامعـــة بابـــــل / كلية العلوم
قســـــم علـــوم الحيـــــاة

دراسة جزيئية ومناعية لفايروس تي اللمفاوي البشري من النوع الأول في اورام الدماغ للمرضى العراقيين

اطروحة مقدمة إلى
مجلس كلية العلوم – جامعة بابل
وهي جزء من متطلبات نيل شهادة الدكتوراه فلسفة في العلوم /علوم الحياة

من قبل
عطاء خليل حسين جاسم الشمري
بكالوريوس علوم في التقانة الاحيائية / جامعة بابل (٢٠١٥)
ماجستير علوم في الاحياء المجهرية/ جامعة القادسية (٢٠١٨)

بإشراف
الاستاذ الدكتور
شاكر حماد محمد حسن العلواني

١٤٤٥ هـ ٢٠٢٣ م