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BIOCHEMICAL AND IMMUNOLOGICAL STUDY IN IRAQI PATIENTS WITH BRAIN TUMOR

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AMANI SALAM MERZAH ABBOOD

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BIOCHEMICAL AND IMMUNOLOGICAL STUDY IN IRAQI PATIENTS WITH BRAIN TUMOR

By Amani Salam Merzah ABBOOD

April 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

BIOCHEMICAL AND IMMUNOLOGICAL STUDY IN IRAQI PATIENTS WITH BRAIN TUMOR

Amani Salam Merzah ABBOOD

Master of Science in Chemistry

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April 2022

From November 2021 to February 2022, researchers at Al-Hussein Hospital in Karbala, Iraq, will investigate the prevalence of bacteremia and immunological markers in patients with brain tumors. 50 patients with brain tumors (27 males, 23 females) and 30 healthy people as controls. Both patients and controls had blood drawn. Blood urea, creatinine, alkaline phosphate, and CKBB were measured by Fujifilm, and interleukin-12 (IL-12) by ELISA. The findings indicated that the most vulnerable age group was 1-10 year olds, with a 26% infection rate compared to other age groups. The average urea level in patients was 29.74 mg/dl compared to 28.21 mg/dl in healthy controls, indicating no significant difference between the two groups, unlike creatinine, which was higher in patients 0.6 mg/dl compared to 0.4 mg/dl in their parents, with a p 0.01 significant difference. Also, the level of Alkaline Phosphatase (188.22 U/L) was not substantially different from the healthy (187.76 U/L), nor was the level of CK-BB ($p>0.005$). Interleukin-12 levels in patients are lower than in healthy controls.

2022, 45 pages

Keywords: Brain, Biochemical alterations, CK-BB, Alkaline phosphatase, IL-12

ÖZET

IRAKLI BEYİN TÜMÖRLÜ HASTALARDA BİYOKİMYASAL VE İMMÜNOLOJİK ÇALIŞMA

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Nisan 2022

Kasım 2021'den Şubat 2022'ye kadar, Irak, Kerbela'daki Al-Hussein Hastanesi'ndeki araştırmacılar, beyin tümörlü hastalarda bakteriyemi ve immünolojik belirteçlerin prevalansını araştıracaklar. Beyin tümörlü 50 hasta (27 erkek, 23 kadın) ve kontrol olarak 30 sağlıklı insan. Hem hastalardan hem de kontrollerden kan alındı. Kan üre, kreatinin, alkalin fosfat ve CKBB Fujifilm ile ve interlökin-12 (IL-12) ELISA ile ölçüldü. Bulgular, en savunmasız yaş grubunun diğer yaş gruplarına kıyasla %26 enfeksiyon oranıyla 1-10 yaş grubu olduğunu gösterdi. Hastalarda ortalama üre seviyesi, sağlıklı kontrollerde 28.21 mg/dl'ye kıyasla 29.74 mg/dl idi; bu, kreatinin aksine, hastalarda 0.6 mg/dl'ye kıyasla daha yüksek olan kreatinin'in aksine, iki grup arasında anlamlı bir fark olmadığını gösteriyor. ebeveynler, p 0.01 anlamlı bir farkla. Ayrıca Alkalin Fosfataz düzeyi (188.22 U/L) sağlıklı olandan (187.76 U/L) önemli ölçüde farklı değildi ve CK-BB düzeyi de ($p>0.005$) değildi. Hastalarda interlökin-12 seviyeleri sağlıklı kontrollere göre daha düşüktür.

2022, 45 sayfa

Anahtar Kelimeler: Beyin, Biyokimyasal değişiklikler, CK-BB, Alkalin fosfataz, IL-12

PREFACE AND ACKNOWLEDGEMENTS

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Amani Salam Merzah ABBOOD
Çankırı-2022

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LIST OF SYMBOLS

%	Percent
µL	Microliter
L	Liter
mg/dL	Milligrams per deciliter
mL	Milliliter
nm	Nanometre
ºC	Celsius
pg/mL	Picogram per milliliter
U/L	Units/litre

LIST OF ABBREVIATIONS

ADP	Adenosine Diphosphate
ALP	Alkaline Phosphatase
APCs	Antigen- Presentating Cells
ATP	Adenosine Triphosphate
BUN	Blood Urea Nitrogen
CK-BB	Creatine Kinase Brain Type
CKD	Chronic Kidney Disease
Cr	Creatine
CTLA4	Cytotoxic T-Lymphocyte Associated Antigen -4
DCs	Dendritic Cells
GFR	Glomerular Filteration Rate
IL-12	Interleukin -12
TGF-b1	Transforming Growth Factor -b1

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1. INTRODUCTION

As predicted, the human brain has the same amount of neurons as a monkey brain of comparable size (Marino *et al.* 2007). More than 80 percent of brain mass is ascribed to an overdeveloped cerebral cortex that contains 100 billion neurons and ten times as many glial cells. Countless neurons and non-neuronal cells were discovered therein (Herculano-Houzel 2009).

When cells multiply and replicate uncontrollably in an intracranial tumor, commonly known as a brain tumor, they seem to be unchecked by the regular mechanisms that regulate cells. Primary and metastatic brain tumors are the two most frequent forms, however there are more than 150 other varieties. As a general rule, primary brain tumors grow from inside the brain's tissues or its immediate surroundings. Primary tumors in the brain, whether glial or non-glial (forming on or in the brain's nerves, blood vessels, or glands), are either benign or malignant. Metastatic brain tumors are cancers that originate elsewhere in the body (such as the breast or lungs) and then spread to the brain through the circulatory system. Cancerous tumors that have spread to other parts of the body are known as metastatic tumors (Wang *et al.* 2020).

Many biochemical changes occur in individuals with brain tumors, which indicate the extent to which the tumor has spread and how severe it is. There are biochemical and immune factors that are affected by the tumor, including urea , creatine, Alkaline Phosphatase , CK-BB and IL-12. Regulatory and excretory activities are the primary responsibilities of the kidney in humans. The kidney maintains the body's water level, plasma electrolyte composition, and plasma pH. In addition, the kidney offers a route for the excretion of water-soluble, low-molecular-weight chemicals that are found in the bloodstream. Protein breakdown products like urea and creatinine are examples, (William and Macnab, 2009).

Because of its name, alkaline phosphatase (EC3.I.3.1.) is an enzyme that breaks down phosphate monoesters in acidic environments. Intestinal ALP, Placental ALP, Germ cell

ALP, and tissue nonspecific (L/B/K) ALP are the four isozymes of alkaline phosphatase based on where it is expressed in the body. Toward the end of chromosome 2's long arm, it is located (Sharma *et al.* 2014).

The brain releases CK-BB, a creatine kinase isoenzyme exclusive to brain tissue, into the bloodstream. CK-BB has been shown to elevate rapidly after a mild or moderate brain injury and rapidly decrease over the next 6 hours. Indeed, it seems to be sensitive and specific to injury (e.g., cerebral contusion) and not to any other acute neurologic disorders such as epilepsy or severe headaches (Nordby and Urdal 1982). Although CK-BB does exist in other organs, injury to the brain appears to release much larger quantities of the isoenzyme than injuries to other organs and therefore remains a relatively specific marker for brain injury (Skogseid *et al.* 1992). When compared to neuron-specific enolase, a brain injury specific marker, in patients who had sustained minor-to-severe head injuries, CK-BB exhibited a strong correlation (r = 0.87) (Carr *et al.* 2009)

Malignant tumors in these patients are linked to the development and progression of interleukin (IL)-12, for example. IL-12 is considered a potential cancer immunotherapy approach due to its strong effect on the establishment of an anticancer immune response. IL-12 has been shown in multiple studies to successfully kill cancer cells both in vitro and in vivo. Tumor-associated macrophages' tumor-supportive activities may be drastically reduced by IL-12's antiangiogenic and antiangiogenic properties (Hong *et al.* 2022).

Because of the lack of studies on this subject in Iraq, we believe it is necessary to study this pathological condition, which has had an impact on its spread in Iraq over the past two decades.

1.1 Aim of Study

The study aims to study the biochemical and immunological factors of brain tumor disease in Iraq.In this study, biochemical and immunological changes occur in brain cancer patients were studied and compared with healthy patients. The study's aim is accomplished by:

1. Assessment kidney function
2. Assessment levels of alkaline phosphatase.
3. Assessment level of creatinine kinase BB.
4. Evaluation IL-12 levels. Furthermore to know the reasons that led to the spread of this type of cancer in children more than the other age groups.

2. LITERATURE REVIEW

2.1 Brian Tumor

There are a number of different types of brain tumors, each with a distinct biology, prognosis, and therapy; these tumors are best referred to as "intracranial neoplasms," as some of them don't originate in the brain tissue (e.g., meningiomas and lymphomas) (Wang *et al.* 2020).

Tumors of the brain are the greatest cause of cancer death in children, and despite breakthroughs in surgery and adjuvant medication, treatment is still tough. Cells in brain tumors exhibit a wide range of neural lineage markers and are often morphologically varied (Singh *et al.* 2003).

Brain Tumor Types There are several factors to take into account while considering brain tumors, including their origin and location inside the brain. No matter how many neurons are present, they are incapable of recreating themselves. Therefore, the neoplastic alterations that lead to a tumor are very uncommon in initial neural malignancies. glial cells, the brain's structural and metabolic supporting cells, are numerous and perform a variety of roles, including safeguarding neurons, enhancing their efficiency, and ensuring their sustenance and electrical stability. Each of these tumors may be either benign or malignant, depending on the kind of cell from which it was formed, and each of these tumors can be either benign or malignant. More than half of all initial brain tumors are gliomas. Trocytoma is the most prevalent type (Barton *et al.*1990). There are many types of oligodendroglioma and malignant glioblastoma. The meninges that surround the brain and spinal cord protect the brain and are crucial for sustaining cerebrospinal fluid routes. Meningiomas are a frequent kind of tumor that develops in the brain and spinal cord. They are nearly invariably benign. Indentations caused by these tumors frequently indent the brain, resulting in symptoms such as headaches and dizziness (Edward *et al.* 1993).

It is possible for a brain tumor to generate both localized and systemic symptoms. Headaches, nausea, vomiting, and a sixth-nerve palsy are all generalized signs of elevated intracranial pressure. The tumor's intracranial placement is indicated by specific symptoms and indicators, such as hemiparesis and aphasia. The kind of tumor determines the frequency and duration of symptoms. It is more common for a high-grade glioma to develop quickly progressing hemiparesis (D and Ngelis 2001).

2.2 Kidney Functions

Regulatory and excretory activities are the primary responsibilities of the kidney in humans. The kidney maintains the body's water level, plasma electrolyte composition, and plasma pH. In addition, the kidney offers a route for the excretion of water-soluble, low-molecular-weight chemicals that are found in the bloodstream. Protein breakdown products like urea and creatinine are examples, but so are other substances with comparable physicochemical properties (William and Macnab 2009).

2.2.1 Urea

The liver produces urea, a main nitrogenous end product, which is transported throughout intracellular and extracellular fluids and the blood during protein and amino acid degradation. The kidneys' glomeruli remove urea from the blood and partially reabsorb it with water (Gowda *et al.* 2010). Serum urea concentration is a key factor in determining the most common clinical indicators used to assess renal function. Increased blood urea nitrogen–creatinine ratio may be used to diagnose acute renal failure and pre-renal disorders (Rosner and Bolton 2006). The overproduction rate of urea clearance is dependent on non-renal variables including food and urea cycle enzymes, making it a poor indication of glomerular filtration rate (GFR). An increased level of blood urea nitrogen (BUN), which may indicate renal illness or failure, urinary tract blockage due to a kidney stone, congestive heart failure, or any of these disorders, is connected to dehydration, fever, shock, and gastrointestinal bleeding. Having a high BUN level may arise due to consuming a lot of protein-rich meals in the late stages of pregnancy or as a side effect of that diet. Over 100 mg/dL of BUN indicates renal

damage, however lower BUN values are reported in conditions of fluid excess. There is a correlation between low levels of oxytocin, painkillers, hunger, and anabolic steroid use (Gowda *et al.* 2010).

2.2.2 Creatinine

Depending on muscle mass, the body produces a relatively consistent amount of creatinine, which is the breakdown product of creatine phosphate. The creatinine level is a typical indicator of renal function and is often tested. In men, test valve creatinine clearance ranges from 110-150 ml/min to 100-130 ml/min, which is considered normal (Gowda *et al.* 2010). The glomerular filtration rate may be calculated using serum creatinine concentration, as suggested by the National Kidney Disease Education Program (Greg *et al.* 2005). Creatine clearance tests are used to monitor the progression of kidney disease. Renal failure is usually suspected when serum creatinine rises over the upper limit of the so-called "normal" range. In chronic renal failure and uremia, both the glomeruli and the tubules gradually diminish creatinine excretion (Gowda et al. 2010).

As the illness progresses, patients with cancer may suffer from a variety of side effects, including acute renal damage (AKI). Acute kidney injury (AKI) is linked to cancer (Campbell *et al.* 2014). In these patients, AKI is caused by TLS, the use of antimicrobials, chemotherapeutics, and contrast media, direct invasion of the genitourinary system by cancer cells, or multiorgan failure following sepsis and other morbidities, such as pneumonia. Researchers found out about this (Park *et al.* 2019). Because of the risk of AKI during cancer treatment, several therapeutic medications may have to be changed, or even eliminated. This might have a severe influence on the patient's outcome. Acute kidney injury (AKI) in a cancer survivor is a recognized risk factor for the development of chronic kidney disease (CKD). According to the authors' assertions (Chawalas *et al.* 2014). Acute kidney injury (AKI) in cancer patients has been studied extensively. An estimated 25.8 percent of 37 267 cancer patients were diagnosed with acute kidney damage (AKI) within one year after diagnosis, while their 1-year total AKI incidence rate was 17.5. A recent study found that more than a twelfth

of cancer patients admitted to a hospital had AKI. More information may be found at (Salahuddin *et al.* 2013). There is a lack of data on the prevalence of AKI in pediatric cancer patients. AKI has been reported in between 11% and 84% of individuals with hematologic malignancies in the few studies that have been done. Children with cancer are more susceptible to acute kidney injury (AKI), which has been linked to long-term renal damage (Park *et al.* 2019).

2.2.3 Acute renal failure in cancer patients

Among cancer patients, ARF is a significant condition that increases morbidity and death. Patients with ARF typically have to take lower than recommended dosages of chemotherapy and supportive medications like morphine compounds and antibiotics, which might have a negative impact on their health. Cancer patients who have undergone IONed CT contrast chemicals, nephrotoxic chemotherapy or HPCT, TLS, post-renal blockage or malignant infiltration are more prone to develop ARF. ARF may be prevented by identifying patients who are most at risk and implementing preventative treatments. As a major consequence in cancer patients, acute kidney damage (AKI) has a high morbidity and mortality (Campbell *et al.* 2014). It's possible that cancer therapy may be unsuccessful if the patient has AKI that requires dialysis. in the case of individuals with AKI (Campbell *et al.* 2014).

2.3 Alkaline Phosphatase

An alkaline phosphatase (ALP) is a plasma membrane-bound glycoprotein with the EC 3.1.1.3.1 orthophosphoric monoester phosphohydrolase (alkaline optimal) as its active site (Tsai *et al.* 2000) Most of these enzymes can be found in prokaryotic and higher eukaryotic organisms save for a few higher plant species (Sharma *et al.* 2012). Zinc-containing metalloenzymes (ALPs) expressed by several genes in mammals perform their function as dimeric molecules. In order for an enzyme to function, it must include two Zn2? and one Mg2? ions in the active site. The ALP monomer's structure and the interactions between smaller units are also greatly influenced by these metal ions (Sharma *et al.* 2014).

Membrane-bound metalloenzyme alkaline phosphatase is made up of many isoenzymes. Glycoproteins, the building blocks of each isoenzyme, are encoded by distinct gene loci (Weiss *et al.* 1988). The placental, placental-like, and intestinal ALP isoenzymes are made by three ALP genes on chromosome 2q34–37, and their expression is tissue-specific (PALP, PLALP and IALP respectively). On human chromosome 1, distal short arm bands p34–p36 contain the L/B/K ALP gene, the gene's last and final location. 1 encodes a wide variety of protein families (Weiss *et al.* 1988). Although there are secondary ALP enzymes in every cell of the body, they are concentrated in the liver, skeletal muscles, and kidneys due to differences in TNSALP glycosylase isoforms formed by tissue-specific glycosylation (Moss 1992). ALP tissue-nonspecific (TNSAAP) is another name for liver, bone, and kidney (Whyte *et al.* 1995). The L/B/K gene is at least five times longer than the other three genes. The number of introns in the L/B/K ALP gene is significantly greater than that of the other ALP genes. Genes for intestinal, placental, and placenta-like tissues may have smaller introns (74–425 bp). Unlike other genes, ALP's whole cDNA sequence is known, and it contains 12 exons instead of the other genes' A total of 11 coding exons, from exons 2–12. At the conclusion of exon 50, a brand new exon has been added. The non-coding region in human DNA is 25 kilobases long. Exons 1 and 2 are separated by at least 25 kilobases of DNA. As a result, at least 50 kilobases (kb) of DNA make up the complete gene (Sharma *et al.* 2014)

2.3.1 How alkaline phosphatase works in the body

Serum measurements of liver and bone alkaline phosphatase activity have long been used in regular medical diagnosis. Data on healthy persons of various ages, as well as findings from various disorders, are presented. At least half of the activity in healthy persons comes from the bone alkaline phosphatase. From 20 to 140 U/L, alkaline phosphatase levels may be seen in the bloodstream. Numerous disorders of the bones, liver, and other organs have been related to elevated levels of the enzyme alkaline phosphatase (Emanual *et al.* 1956). Biliary obstruction may be indicated by elevated ALP values. Children and expectant mothers have much greater levels than the general population (Stigbrand *et al.* 1982). There are fewer cases with ALP levels that are lower

than normal. Low alkaline phosphatase levels may be caused by cretinism and achondroplasia in children and adults, malnutrition, magnesium deficiency and hypothyroidism in adults, acute enteritis and pernicious anemia in children and adults, chronic myelogenous leukaemia, and Wilson's disease in adults. A decrease in alkaline phosphatase has also been shown to occur with some medications, such as oral contraceptives (Schiele *et al.* 1998). All tissues in the body are almost completely devoid of L/B/K ALP in the most severe instances (kTsai *et al.* 2000). ALP ectopic expression has been linked to a wide range of human malignancies. Changes in the ALP gene's expression have suggested the notion that ALP isozymes are implicated in carcinogenesis (Sharma *et al.* 2012). Meningiomas have a mutation that renders the ALPL tumor suppressor gene inactive in the homozygous state. A study by (Isolde *et al.* 1997). Breast cancer patients have been shown to have a higher level of ALP activity (Sharma *et al.* 2014).

2.4 CK-BB

Cells and tissues throughout the body produce creatine kinase, an enzyme that plays a role in a variety of processes. Creatine is converted into phosphocreatine and adenosine diphosphate (ATP) by CK, which uses adenosine triphosphate (ATP) (ADP). If the CK level increases, it is a symptom of muscle damage. Rhabdomyolysis, myocardial infarction, dystrophic myocarditis, myositis, and myocarditis are all symptoms of the damage caused by this condition, as are malignant hyperthermia and neuroleptic malignant syndrome. The incidence of increased CK levels was considerably greater in patients aged 45 years or older than in those under 45 years of age. Telbivudine-induced CK increases typically appeared at least 21 months after treatment and were generally asymptomatic and short-lived. These findings were in line with those of the GLOBE research (Zou *et al.* 2011). Patients who engaged in high-intensity exercise were found to have elevated CK levels, which reduced without interruption of their telbivudine treatment when they stopped exercising. Patients were instructed not to engage in any high-intensity, long-duration or weight-bearing activity in this trial. Nevertheless, CK elevations were shown to be predicted independently by being younger and male. It was hypothesized (Wu *et al.* 2010) that HBeAg might influence disease development by

reducing the production of inflammatory cytokines and IFN genes, as well as by decreasing the activity of NF-jB signaling and the IFNß promoter (in young boys) (Wu *et al.* 2010). Toxins and proinflammatory genes may be activated by NF-jB activation after exercise, which may lead to muscle damage (Zou *et al.* 2011).

2.4.1 CK-BB structural homology modeling

To create a 3D model of CK-BB, John and Sali employed MODELLER9v1 (John and Sali 2003), which includes 381 amino acids (Rodriguez et al. 1998). The query sequence is compared to known homologous structures, and an all-atom model is constructed as a result. We found structures that were homologous to CK-BB. The structure of bovine retinal creatine kinase A was shown to be a good model for the protein's structure (PDB entry: 1g0w). Comparative sequence alignments of the genes 1g0w and CK-BB were constructed using MODELLER's ALIGN2D tool. An very accurate 3D model of CK-BB was built using sequence alignment. The study by (Lü *et al.* 2010)

The mitochondria and other organelles, such as the nucleus, rely on CK enzymes to transport energy back and forth. Overexpression of the human brain CK gene in COS-7 cells was cloned. His-65 and Pro-66 were subsequently deleted, since they were shown to be in the midst of a flexible loop in mitochondrial and cytosolic CK by X-ray crystallography (see below) (Mourad-Terzian *et al.* 2000).

The CK-BB measurement technique was improved by Abbott and Lott after previous investigations (Lott and Heinz 1982). In serum samples, 2-mercaptoethanol and EDTA have been shown to revive inactivated CK-BB. In individuals with diverse types of cancer, the detection rate of CK-BB increased from 34% to 78% when these and other parameters were tweaked. Hematologic malignancies had the least rise in detection rates, whereas solid tumors exhibited the biggest. Only two of the 5Q cancer patients were positive for CK-BB in normal serum, despite the presence of CK-BB in normal serum. (Abbott and Lott 1984)

2.5 IL-12

As a reaction to microbial infections, dendritic cells produce anti-inflammatory mediators such as IL-12 (Arase *et al.* 2002). Antigen presentation cells produce more IL-12 in response to the production of IFN-g by T cells triggered by IL-12, leading to the development of the TH1 response. Natural killer cells, in response to IL-12, may also release IFN-g (Vignali and Kuchroo 2012).

2.5.1 Roles of IL-12 in the body

T and NK cells are two of IL-12's primary targets in the body (Kaplan *et al.* 1996). IFN-g production is the most notable aspect of innate and adaptive immune responses. Stimulating cells with the unrelated cytokine IL-18 results in a synergistic increase of IFN-g production. The IL-12 and IL-12R interaction phosphorylates the promotor for STAT4 in the IFN-g gene, which subsequently binds to STAT4. When IFN-g is activated in macrophages, it produces an enzyme called NO synthase, which causes the release of reactive nitrogen species. As a consequence, phagocytic activity and inflammation in the area are both boosted (Zundler and Neurath 2015)

2.5.2 Cancer

Immune suppression by Tregs was completely reversed when recombinant IL-12 and CTLA4 inhibitors were used. Glioblastoma patients with a high percentage of Tregs have a weaker immune response because of this (Berg *et al.* 2013). There is a strong correlation between IL-12's anti-tumor activity and its ability to change substantially depending on the tumor type and experimental conditions, according to these findings. In the late 1990s, the first clinical studies using recombinant IL-12 in patients were launched, and the findings were mixed at best (Bajetta *et al.* 1998). As a result, newer and more complex approaches of boosting IL-12 activity have been developed in the meanwhile. For example, exogenous IL-12 delivery, genetically modified cells that release IL-12 or the co-administration of IL-12 with tumor antigen vaccines have

resurrected the use of IL-12 as a cancer treatment strategy (Lasek *et al.* 2014). As of May 31st, 2015, www.clinicaltrials.gov listed several trials that were still in progress, including one involving a human IgG1 antibody fused to two IL-12 molecules, another with the cancer-fighting adenoviral vector, and a third involving intratumoral injection of an IL-12-encoding DNA plasmid followed by electroporation. According to the findings of these trials, IL-12 immunotherapy may have a future in the treatment of human cancer, at least in certain cancer types and patient subgroups (Zundler and Neurath, 2015)

2.5.3 IL-12: A Look at Its Biology

There are several strategies to use cytokines in cancer treatment. Cytokines are one of the main actors in modulating immune responses. With regard to this, the immunomodulatory and antitumor-inducing factor (IL-12) appeared to be the most powerful anti-tumor agent. In 1989, IL-12 was shown to be an NK cell stimulator factor that has a broad variety of biological effects on peripheral blood lymphocytes, including NK cell proliferation (Kobayashi and colleagues 1989). Lymphocytes such as macrophages and B cells, which have been activated by a Toll-like receptor, generate the majority of this cytokine. As an example (Trinchieri *et al.* 1993). This cytokine has been shown to promote inflammation and is produced early in the course of an infection. For optimum IL-12 synthesis, signals such as interferon-g (IFN) and IL-15 may be added to IL-12 manufacturing process (Schulz *et al.* 2000). In contrast, TGF-b1 and IL-10 negatively affect IL-12 (Du and Sriram 1998). There are covalent disulfide connections connecting the two heavy (p40) and light (p35) chains that make up Il-12's 70 kDa heterodimer (Schoenhaut *et al.* 1992) P35, which is thought to be required for the generation of physiologically active cytokines by phagocytic cells, is only expressed at low levels. As a result of (Babik *et al.* 2022) This hormone is detected by the IL-12 receptor (IL-12R), a heterodimeric protein composed of two subunits, one in each of the two cells of the body (Tugues *et al.* 2015a).

3. MATERIALS AND METHODS

3.1 Laboratory Instruments

The instruments and tools have been used in this study were listed in Table 3.1.

Table 3.1 Instruments and tools that used

NO	Apparatus	Company/Origin
1	DIMENSION EX2 200	Siemens Healthineers/Germany
2	MINDRAY BS-240 pro	Shenzhen/ China];
3	FUJIFILM DRI-CHEM NX500i	FUJIFILM Corporation/ Japan
4	PST-60HL BIOSAN	Biosan/Lativa Europe
5	COMBIWASH	HUMAN/ European Union
6	HUMAREADER HS	HUMAN/ European Union
7	Centrifuge	Anke, Europe S.A.
8	Jel Tubes	Turkey
9	Pipette	French
10	Series variable volume channel micropipette	Dragon
11	Filter Tipes	Jordan
12	Pendroff tubes	Jordan
13	Timer watch	China
14	Plastic droppers	China
15	Incubator	Memmert / Germany
16	Gloves	Sidra/ China
17	Face mask	Broche/ China
18	Cylinders	Technico/ England

3.2 Biochemical and Immunological Kits

Table 3.2 lists the biochemical and immunological kits utilized in this investigation.

Table 3.2 This investigation made use of biochemical and immunological test kits

NO	Kit name	Company	Origin
1	UREA Urea Assay Kit	Shenzhen	China
2	DIRUI CRE-ENZYME	DIRUI	China
3	ALP Alkaline phosphatase Kit	Shenzhen	China
4	FUJIFILM CK-BB Kit	FUJIFILM	Japan
5	Elabscience IL-12 ELISA Kit	Elabscience	Texas,United States

3.3 Blood Samples Collection.

Blood samples were collected from patients with brain tumor (50 samples and with 30 samples from healthy persons), where the blood sample was withdrawn from patients and healthy persons. Samples have been placed in a gel tubes and then centrifugation to obtained serum. The withdrawal of serum and its placement in the Pendroff tubes to be use in biochemical and immunological tests required.

3.3.1 Urea assay

Principle of the Assay

Urine, serum, plasma, cell lysates, and tissue homogenates may all be used with the Urea Assay Kit to determine urea concentrations. Microtiter plate samples are compared to urea standard concentrations of established concentrations. Enzyme urease hydrolyzes ammonia and CO2 from urea for 10 minutes in the presence of samples or standards. Ammonia combines with an alkali solution chromogen to generate a blue-green dye. It takes around 30 minutes to read the plate using a typical 96-well optical density microplate reader. High urea concentrations are associated with higher OD readings. Urea standards are used to determine the urea concentration of samples. Up to 50 mg/dL urea, the standard curve is linear.

Normal Rang 15-45 mg/dl

3.3.2 Cre-enzyme

Picric acid method

Principle

Alkaline picrate is formed when picric acid combines with creatinine to generate an orange-colored compound. The level of creatinine in the sample has a direct correlation to the intensity of the resulting color:

Creatinine + Alkaline Picrate→ Orange Colored Complex

Normal Rang 0.4-1.3 mg/dl

3.3.3 Alkaline phosphatase

Principle of assay:

It is a colorimetric test for alkaline phosphatase (ALP) activity in blood and other mammalian samples that is extremely sensitive, simple, direct, and suitable for high-throughput screening (HTS).

Normal Rang 30-317 U/L

3.3.4 Fujıfılm ck-bb kit

Principle of the assay

The Double Antibody Sandwich ELISA method is used in this kit, as stated above. Both antibodies are monoclonal antibodies against CK-BB, whereas the precoated antibody is a monoclonal antibody against CK-BB. After the samples and biotinylated antibodies have been added to the wells of the ELISA plates, they are rinsed with PBS or TBS. Conjugates of avidin and peroxidase are then applied to the wells in the plate. This method uses a TMB substrate as a colorant following a thorough washing of the wells with PBS or TBS. The peroxidase activity of TMB produces a blue product, which

changes to yellow when the stop solution is added (Color Reagent C). A sample's color intensity and the amount of the target analyte it contains are linked. Normal Rang up to 25 U/L

3.3.5 ELISA estimation for IL-12

Principle of IL-12 estimation:

The Sandwich-ELISA method is used in this ELISA kit. As part of the kit, you'll get an ELISA plate pre-coated with an anti-Human PP antibody. The particular antibody is mixed with the standards or samples in the micro ELISA plate wells. An anti-Human PP biotinylated detection antibody is then added to each microplate well, followed by the addition of an Avidin-Horseradish Peroxidase (HRP) conjugate. Everything that isn't needed is swept away. Each well is then filled with the substrate solution. Human PP, biotinylated detection antibody, and Avidin-HRP conjugate will show blue in only those wells that contain the three components. Color changes to yellow when stop solution is added to the enzyme-substrate process. Spectrophotometrically, the optical density (OD) is measured at a wavelength of 450 nm 2 nm. Human PP concentration is directly related to the OD value. If the OD of a sample is compared to the standard curve, it may be determined how much Human Phosphate is in the sample.

3.4 Statistical Analysis

Chi-square tests were used in the analysis of data from SAS 2012. The results were compared using a less important difference (LSD) at a probability level of 0.05, 0.01.

The Data was analyzed in a CRD (Complete Randomized Design) In a practical way 2x2x6. The averages were compared with L.S.D (less important difference) and Chi - square at a probability level of 0.05, 0.01 using S A S 2012 .

SAS 2012. Statistical Analysis System, User,s Guide. Statistical. Version 9.1th ed. SAS. Institute Incorporated Cary. N.C. USA

4. RESULTS AND DISCUSSION

4.1 Results

The current study included 50 patients (27 male and 23 female) diagnosed by the physician with a brain tumor and these patients reviewed to the Warith International Cancer Institute in the city of Karbala, and 30 persons (12 male and 18 female) as control as shown in Table 4.1 and Table 4.2, which represents the number and proportion of patients according to the age group its observed in this study that the category of children aged 1-10 age was the most likely For injury, the rate of infection was 26% compared to the other age groups that were lower than this percentage. Also, through our study, which focused on the study of chemical and immune factors mentioned in the proposal , it was noted that the average level of urea Figure 4.2 and Figure 4.6 in patients was 29.74 mg/dl compared to the healthy 28.21 mg/dl, which indicates There is no significant difference between the injured and the healthy, unlike the creatine level rate, which was higher in patients 0.6mg/dl compared to the 0.4mg/dl in their parents, and the significant difference was at p 0.01, as illustrated in Table 4.3.

As for Alkaline phosphatase Figure 4.3 and Figure 4.7, which was worth 188.22 (U/L) compared to the healthy 187.76(U/L), which does not indicate a significantb difference between patients and the healthy Figure 4.1 and Figure 4.5. As for CK-BB observed that As for CK-BB observed that, there is a significant difference at the level of 0.01 between the healthy and the patients, but for IL-12, there is no significant difference between the healthy and the patients, but there is a mathematical difference where the level of IL-12 in patients is lower than in the healthy. and described in Table 4.4, Table 4.5 and Table 4.6.

Table 4.1 Demographic properties of studing groups

Group / properties	Number	Sex	percentage	P value
Patients	50	M 27 F 23	%54 %46	
Controls	30	M 12 F 18	%24 %36	

Table 4.2 Age distribution of patients and control

Age group	Number	percentage
10-1	13	%26
20-10	10	%20
30-20	7	%14
40-30	8	%16
50-40	6	%12
> 50	6	%12
Total	50	100

Table 4.3 Show concentration of urea(mg/dl) , creatine (mg/dl) and alkaline phosphatase (U/L)

Factor / Group	Urea	Creatine	Alkaline phosphatase
Patients	1.85± 29.74	0.03±0.60	13.96±188.22
Controls	2.42±28.21	0.01±0.41	22.52±187.76
P value	> 0.05 n .s	0.01	n. s

Table 4.4 Show CK-BB(U/L) and IL-12 (pg/mL) levels in both groups

Factor / Group	CK-BB	IL-12
Patients	0.38±6.31	4.60±21.80
Controls	0.49±8.05	4.02±26.09
P value	0.01	n.s

Table 4.5 Show the correlation coefficient for patients

	Creatine	Urea	ALK-PH	CK-BB	IL-12
Creatine	1.000	0.052 n.s	0.28 - 0.04	0.02-n.s	0.17-n.s
Urea	0.052 n.s	1.00	0.18 n.s	0.05 n.s	0.108 n.s
ALK-PH	0.28 -0.04	0.18 n.s	1.00	0.22-n.s	0.25 n.s
CK-BB	0.02-n.s	0.05 n.s	0.22 n.s	1.00	0.008 n.s
IL-12	0.17- n.s	0.108 n.s	0.25 n.s	0.008 n.s	1.00

Table 4.6 Show the correlation coefficient for healthy persons

	Creatine	Urea	ALK-PH	CK-BB	IL-12
Creatine	1.000	0.23 n.s	0.006 n.s	0.09- n.s	0.22- n.s
Urea	0.23 n.s	1.00	0.09 n.s	0.13 n.s	0.006 n.s
ALK-PH	0.006 n.s	0.09 n.s	1.00	0.18- n.s	0.18 n.s
CK-BB	0.09- n.s	0.13 n.s	0.18- n.s	1.00	0.05 n.s
IL-12	0.22- n.s	0.006 n.s	0.18 n.s	0.05 n.s	1.00

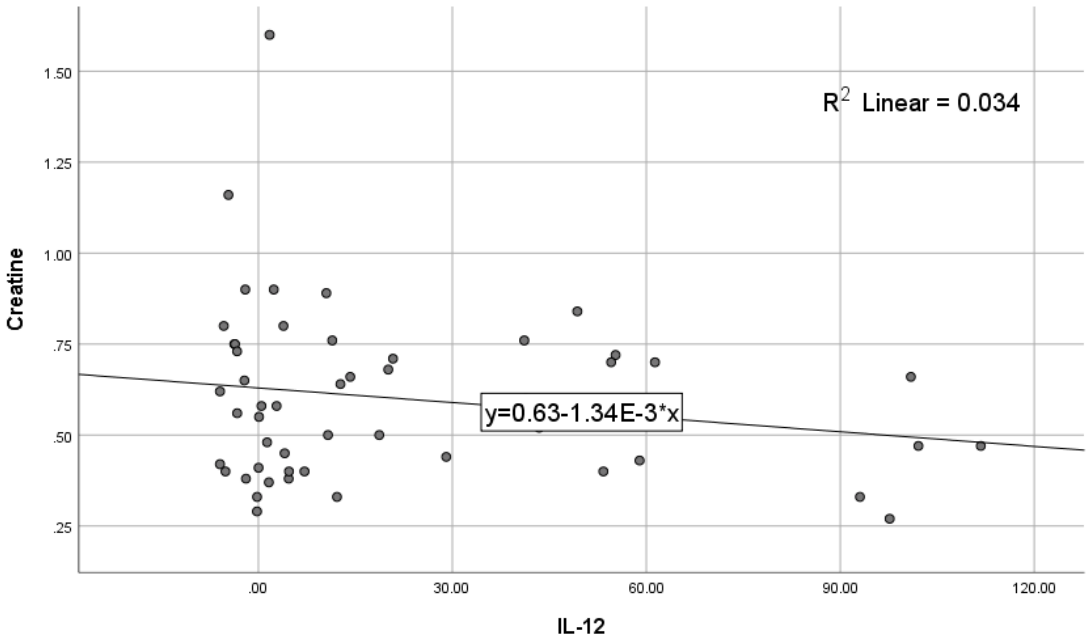

Figure 4.1 The quantitative relationship Between Creatine and IL-12 for patients

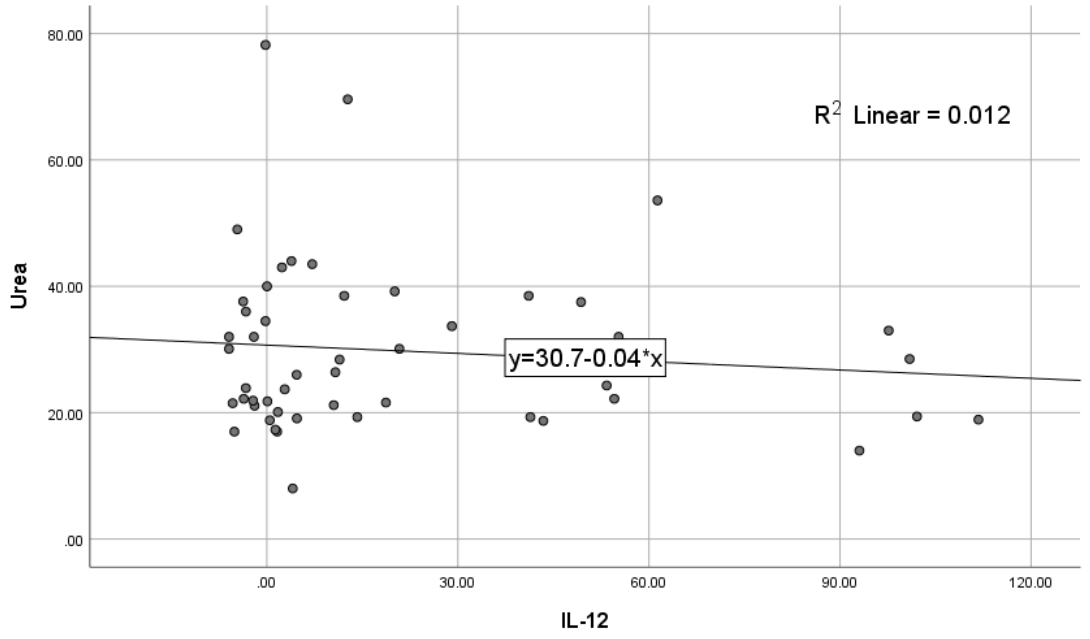

Figure 4.2 The quantitative relationship between Urea and IL-12 for patients

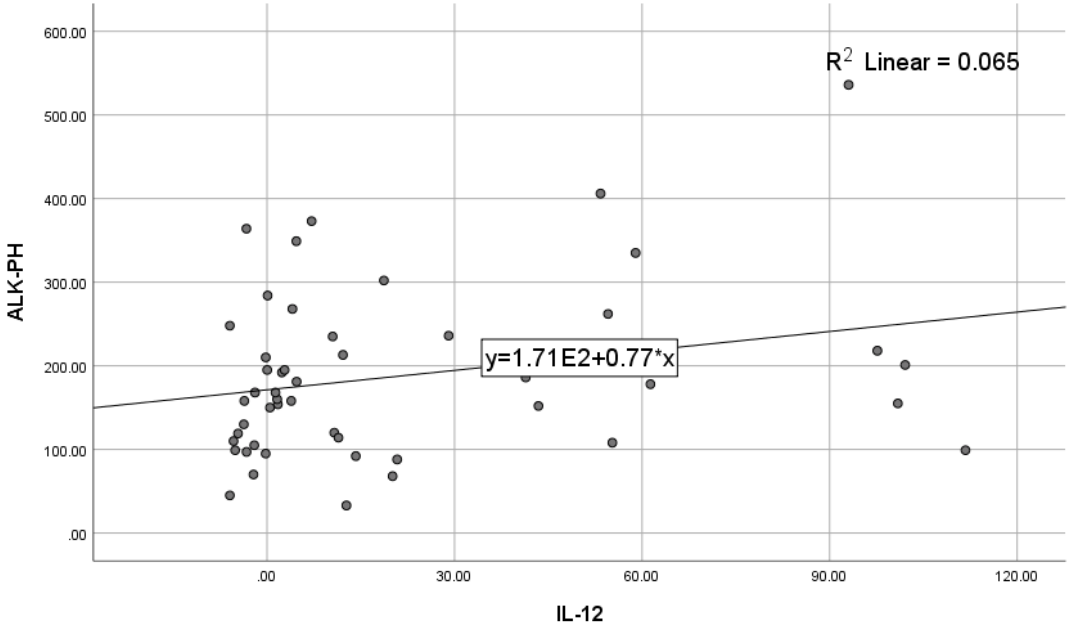

Figure 4.3 The quantitative relationship between the Alkaline phosphatase and IL-12 for patients

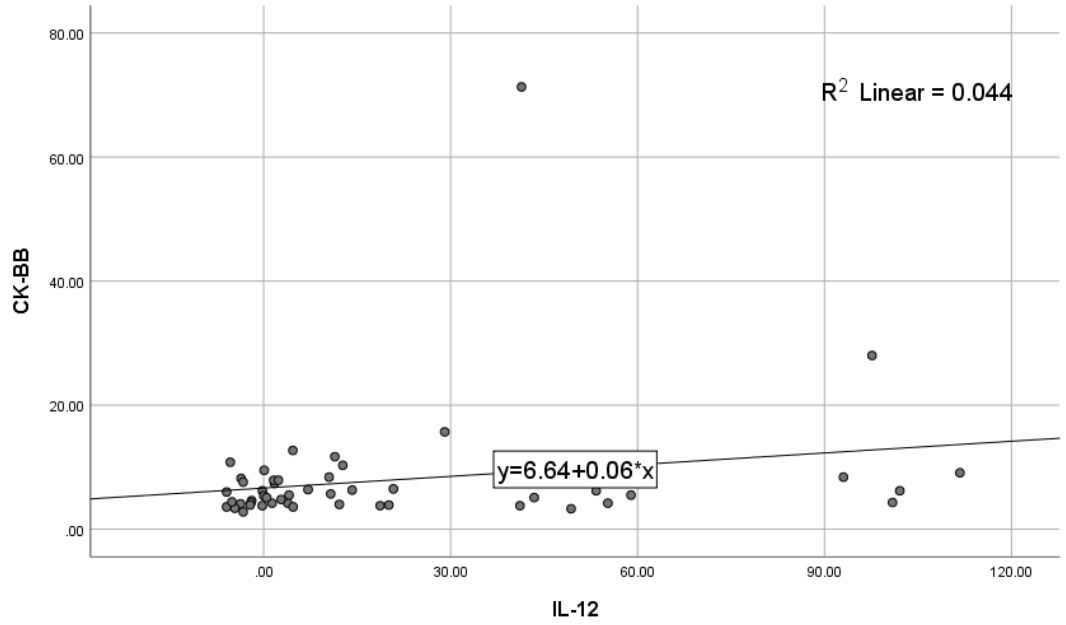

Figure 4.4 The quantitative relationship between CK-BB and IL-12 for patients

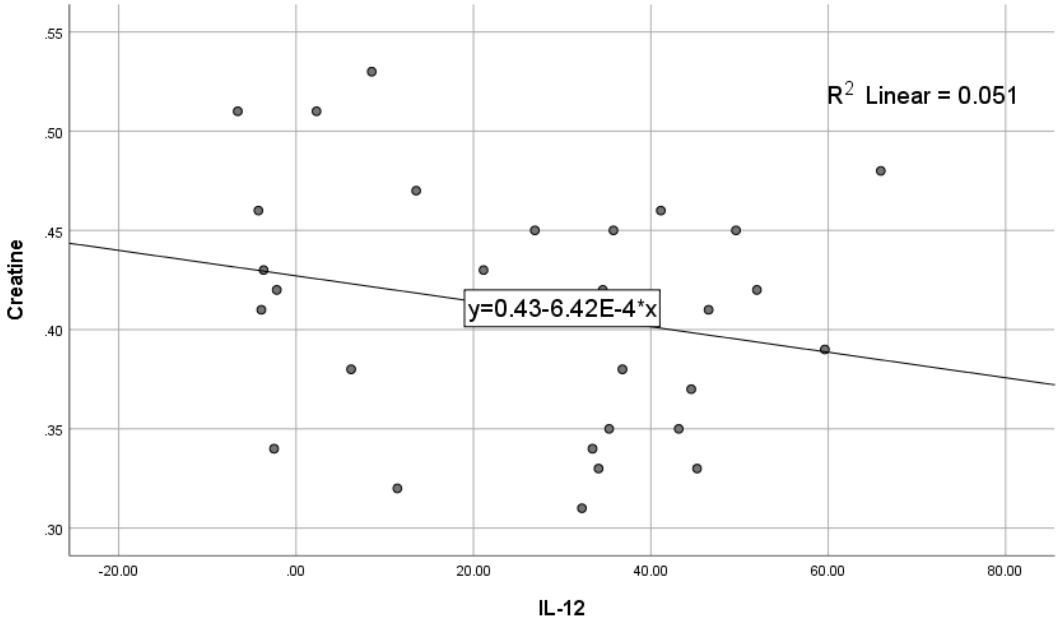

Figure 4.5 The quantitative relationship between Creatine and IL-12 for healthy persons

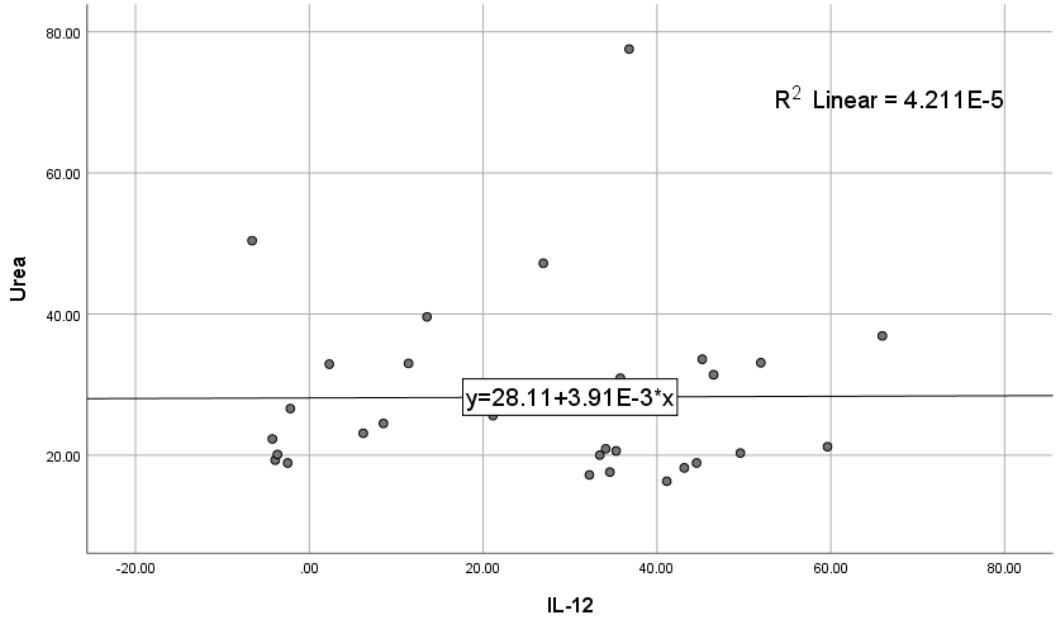

Figure 4.6 The quantitative relationship between Urea and IL-12 for Healthy persons

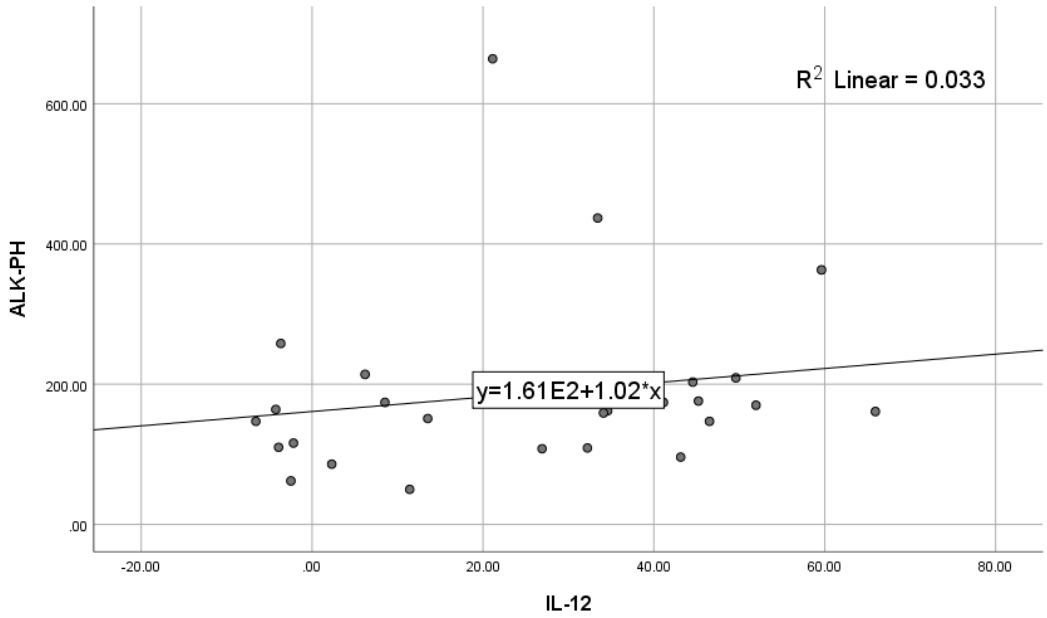

Figure 4.7 The quantitative relationship bwtween Alkaline phosphatase and IL-12 for healthy persons

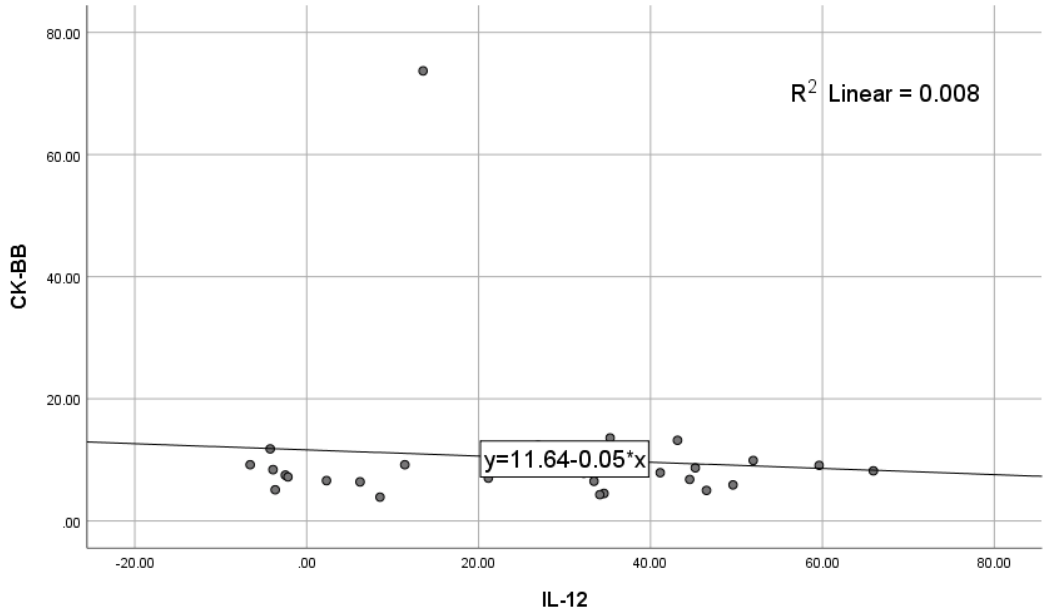

Figure 4.8 The quantitative relationship between CK-BB and IL-12 for healthy persons

4.2 Discussion

4.2.1 Urea and creatinine

Serum creatinine and BUN levels in individuals with traumatic brain injury are examined in detail in this research. There were 50 patients who satisfied the inclusion requirements, as determined by the study's findings. An initial average of 0.60 mg/dl of creatinine in the blood. Blood Urea was found to be on the average at 29.741.85 mg/dl. Serum Creatinine and BUN data indicate that there has been no significant change in the level of creatinine in the blood.

The study was conducted by looking at data of patients diagnosed with brain injury with increased intracranial pressure. The most common brain injury was severe brain injury in all of the monitoring studies conducted. The age group with the highest brain injury was the age group of 16 to 39 years, although patients in the 40-60 age group. Some researchers reported that the risk of acute renal failure due to increased age, so the kidneys are reduced due to acute stress. (Pérez-Pérez *et al.* 2002)mentioned that patients with old age have a higher risk of acute renal failure. Several studies have shown that the risk of acute kidney damage increases with age, hence the kidney's ability to withstand acute stress decreases. Patients with a brain injury were more likely to suffer from acute renal damage if they were older, according to the same study (Dziedzic *et al.* 2003). Mannitol patients who are older are more likely to suffer from acute renal failure, according to previous research (Kim *et al.* 2014). This lends credence to the findings of our investigation. It was found that creatinine was one of the substances generated by organ metabolism that was filtered out of the blood by the glomerulus, whereas tubule secretion was so low that it could be ignored. As a result, creatinine was very beneficial in the assessment of the glomerulus.

Correlation Coefficient: The association between creatine and other factors studied (Urea, Alkaline phosphatase,CK-BB,IL-12), where there is no significant difference except that the association with alkaline phosphatase inversely where the increase of creatine decreases the alkaline phosphatase where the result was different for the

healthy where the association between all the factors studied was no significant difference except alkaline phosphatase the correlation was directly unlike in patients (reverse) as shown in Table 4.5 and Table 4.6.

Creatine Figure 4.1 and Figure 4.5: Creatine is affected by IL-12 by 0.03 percent in patients, while creatine is affected by IL-12 in healthy persons by 0.05. This means that the extent to which IL-12 is affected by creatine is low.

Urea is the largest nitrogen product secreted by the kidneys derived from diets and endogenous proteins that have been filtered by the glomerulus and partially reabsorbed by the tubules. Urea gets more reabsorbed in a state where urine is slow or disturbed (dehydration) (Sari and Wahyuhadi 2020).

By studying the correlation coefficient between urea and other factor studied, they were all found to be non- significant difference in patients as well as in the healthy, which indicates that the rate of urea is not affected by the pathological condition (brain cancer) if compared to healthy people through the results obtained from our as shown in Table 4.5 and Table 4.6.

Urea Figure 4.2 and Figure 4.6: By observing the regression equation, it was found that the determining factor(R^2) in patients was 0.012,while the determining factor in healthy people was $4.21x\ 10^{-5}$, which indicates that the effect of urea at the level of IL-12 in healthy persons is much lower than in the patients.

4.2.2 Alkaline Phosphatase

There is a broad range of AP levels in the blood of different sexes and ages that are considered normal, though (Li *et al.* 2018). Due to the fact that their bones are still developing, children have naturally high amounts of AP. The typical range of Bnormal AP levels is used by doctors and researchers instead of a specific figure. The majority of

the time, aberrant AP levels indicate that the body's homeostasis is off balance and needs to be addressed.

In-depth analysis of clinical samples Total AP enzyme activity, rather than total AP protein content, is measured using AP assays, which are often used in medical laboratories.

Serum AP concentration was shown to be associated with functional outcome in patients with different forms of brain injury, whereas elevated TNAP was found to be negatively associated with cognitive performance (Vardy *et al.* 2012). In individuals with brain tumors, pneumocarcinoma meningitis was more prevalent, and their CSF AP levels were found to be greater than in control patients with epilepsy and stroke (Lampl *et al.* 1990). Patients with non-traumatic cerebral hemorrhages were shown to have elevated liver AP levels (Meythaler *et al.* 1998).

Observed that elevated serum AP levels may emphasize the brain-peripheral immunological interactions during stroke and related acute ischemic stroke to liver enzyme production (Muscari *et al.* 2014). Hyperintensities in significant areas of white matter in the brain have been associated to increased AP levels in patients who have had an ischemic stroke (Liu *et al.* 2016).

The correlation factor for the alkaline phosphatase and other factors(Urea , Creatine , CK-BB,IL-12) found that the association between the alkaline phosphatase and creatine in patients was significant difference and inversely, but for the healthy, the relationship between the alkaline phosphatase and the creatine was no significant difference, but in a directly for creatine as shown in Table 4.5 and Table 4.6.

Alkaline Phosphatase Figure 4.3 and Figure 4.7: It has been observed that the determining factor(R^2) for the patients 0.06 and for healthy 0.03.which indicates that the effect of alkaline phosphatase at the level of IL-12 was low.

4.2.3 Creatine kinase CK-BB

Brain tumor tends to increase CK-BB levels in the serum, which could be due to damage and leaking out of the enzyme to the circulation. Patients with brain tumors had much higher CK-BB catalytic activity than healthy controls, according to our findings. The findings here are in accordance with earlier research (Schwartz *et al.* 1989). The CK-BB level in the serum samples was considered abnormal when it exceeded 10 μg/L. The source of the raised serum CK-BB is the tumor tissue itself due to cell lysis. A high CK-BB activity in tumor cells may be due to the high cell proliferation, migration, or invasion compared with normal cells. The large tumor cell population re-quires a higher and more rapid energy source com-pared to normal cells. Under aerobic conditions, tu-mor tissues can metabolize more glucose than the normal tissue. In order to meet this large and rapid energy demand, tumor cells use CK-BB activity as one option for their energy supply. Another possible reason for the high CK-BB activity could be the high expression of CK-BB gene in tumor cells compared to normal cells (Matias-Guiu *et al.* 1986).

The CK-BB levels of malignant brain tumor patients were higher than those of benign tumor cases. Similar to our finding, Pan et al. reported a significantly lower CK-BB level in malignant tumor patients than benign ones (Pan *et al.* 2013). As a result of these findings, further investigations have shown a reduction in CK and certain of its isoforms' activity in a variety of cancers (Kinoshita and Yokota 1997, Lehnhardt *et al.* 2005, Onda *et al.* 2006). Low levels of serum CK in breast cancer patients may be linked to the body's immunological response, according to our theory. We are all familiar with how the immune system responds to an infection or sickness in order to protect the body (Vivier *et al.* 2011). As a result, tumor formation, progression, and metastasis are all influenced by the body's immune response, which may lead to treatment failure (Nardo and Coussens 2007, Aaltomaa *et al.* 1992). Serum CK levels may indicate the health of the host's immune system. CK levels are thought to correlate with host immunity to cancer, and it has been postulated that reduced levels of CK contribute to tumor growth. T cell growth and activation are regulated by CK, and this plays a role in the immunological response. CK-BB ectopic expression led to an

increase in ATP levels and an increase in phosphorylation of TCR signaling proteins, according to previous studies. It has been shown that CK-BB may increase the amount of tyrosine in the body. (Pan *et al.* 2013) . Tumor size is considered a highly valuable prognostic factor for brain tumor next to the location and the type of brain cells. Brain tumor patients with larger tumor size lesions are often associated with significantly poor clinical outcomes (M. Chang *et al.* 2017). This result may suggest the involvement of oxidative stress in tumor growth and progression. oxidative stress appeared to promote and regulate brain tumor growth by promoting brain tumor angiogenesis, tumor cell proliferation, and metastasis and by limiting tumor cell apoptosis, which are all essential for tumor growth in size and progression to more aggressive forms and metastasis of existing tumors (Holmgren 1985). We found that a high serum concentration of CK-BB was significantly ($p < 0.001$) associated with brain tumor. This agrees with findings of previous studies suggesting that CK-BB may be used as a marker and may have some predictive value in early diagnosing, grading, and identifying early recurrence of the tumor and in monitoring the efficacy of various therapeutic modalities of brain tumor.

The correlation factor for the CK-BB and other factors(Urea , Creatine , Alkaline phosphatase,IL-12) found that there is no significant difference in patients as well as in the healthy except alkaline phosphatase the correlation was directly in patients unlike in healthy (reverse) as shown in Table 4.5 and Table 4.6.

CK-BB Figure(4,8): It has been observed that the determining factor(R^2) for the patients 0.04 and for healthy groups was 0.008. which indicates that the effect of CK-BB at the level of IL-12 was low.

4.2.4 IL-12

In our study and through the results obtained it was found that the level of interleukin-12 has decreased and this indicates that this cytokinase has an important role in reducing the spread of the tumor as in healthy people it was more than in patients and therefore it can be noted that this cytokinase has a role in the process of the formation and spread of tumor in the human body.

It has emerged during the last two decades as one of the most powerful cytokines in preclinical models for promoting anticancer activity. Macrophages, T lymphocytes, and dendritic cells are all impacted by IL-12 in the tumor microenvironment (Tugues *et al.* 2015b). The cytokine IL-12 has the potential to greatly increase the anticancer immune response in preclinical studies. Immune responses to tumors caused by IL-12 are modulated by the kinds and locations of tumors, as well as the cytokines and effector cells involved. The delivery of IL-12 to the tumor site for therapeutic reasons necessitates new approaches. This cytokine is produced by APCs like dendritic cells (DCs), macrophages, and B cells when triggered by Toll-like receptors (TLRs). Research by Trinchieri and colleagues, 1993 Because of this, infections trigger the release of the proinflammatory cytokine IL-12. According to (Medzhitov 2001), Interferon-g (IFN-g), as well as other amplification signals, may be used (B. X. Ma et al., 1996) IFN-gamma (IL-10) and TGF-b1 (TGF-b1), on the other hand, inhibit the production of IL-12 (Du and Sriram 1998, Tugues *et al.* 2015b).

Cancer cells have been made to continually secrete IL-12 in order to understand how this cytokine causes antitumor immune responses. TSA breast adenocarcinoma and C26 colon carcinoma cells overexpressed with IL-12 underwent tumor suppression upon subcutaneous injection (Cavallo *et al.* 1997, Eisenring *et al.* 2010, Martinotti *et al.* 1993). When it came to the rejection of breast cancer TSA-IL-12 cells, IFN-g secreted by CD8 cytotoxic T cells was all that was needed, as opposed to ILCs in B16 melanoma (Cavallo *et al.* 1997) Tumor rejection in the central nervous system has been attributed in large part to T cells that are sensitive to IL-12 (Vetter *et al.* 2009, Berg *et al.* 2013). Tumor cell type and tumor location, as well as certain effector cell types and cytokines, have an impact on the quality of the immune response to tumors, according to these studies. IFNg-inducible chemokine levels are up and VEGF and metalloproteinase-9 synthesis are down when IL-12 is inhibiting tumor vasculature formation, according to research (Dias *et al.* 1998, Kanegane *et al.* 1998, Mitola *et al.* 2022).

Studies show that IL-12-induced IFN-g is an important tumor growth inhibitor (Tugues *et al.* 2015b, Cancer and York 2004). Even after tumor injection, IL-12's anticancer

effects were still evident, and they were discovered to be partly reliant on CD8 T cells (Tugues *et al.* 2015b)

The supplied IL-12 was shown to activate myeloid cells and promote tumor antigen-specific CD8 T cells, which resulted in tumor regression (Kerkar *et al.* 2011, Lisiero *et al.* 2022, Zhang *et al.* 2009, Pegram *et al.* 2012). IL-12 delivered directly to the tumor site via immunocytokines is a relatively new development. Antibodies specific to the tumor's vascular system or necrotic core DNA have recently been fused with immunocytokines (Halin *et al.* 2002, Sommavilla *et al.* 2010), for example. Necrotic areas inside tumors are of special interest because of the lack of circulation and cell death in solid tumors. Before utilizing antibody-targeted cytokines, a detailed evaluation of the tumor setting is necessary since significant avidity and retention to the targeted tissue are required for good therapeutic effects. Researchers found that targeting CD30 lymphoma cells with dual cytokine–antibody fusion proteins reduced tumor growth more efficiently than doing so with simply IL-12 or IL-2 as a single antigen in their treatment regimen.

IL-12 increases IFN production via activating CD8+ T cells and NK cells, which in turn increases IFN production. Studies have demonstrated that IFN- may kill tumor cells directly, stop the growth of blood vessels, increase the generation of NK cells, CTLs, and macrophages, all while increasing the expression of MHC I and II molecules on the tumor cell's surface. (Bromberg *et al.* 1996, Martini *et al.* 2010, Hayakawa *et al.* 2002, Rosa *et al.* 1986). Systemic injection of therapeutic IL-12, which has shown the ability to ameliorate local immune suppression in preclinical models, has been hampered by substantial inflammation-related adverse effects in clinical studies. To our knowledge, this is the first time that low doses of an antibody fusion protein (NHS–rmIL-12) have been successfully employed to inhibit syngeneic carcinomas with high immunologic activity (Hong *et al.* 2022). IL-12's anti-cancer properties have been repeatedly shown in preclinical carcinoma models (Noguchi *et al.* 1996, Zaharoff *et al.* 2010, Thomas *et al.* 2000). Clinical studies of Il-12 treating advanced cancer patients have been stymied by substantial adverse effects seen in early trials (Lacy *et al.* 2009, Higano *et al.* 1997, Hong *et al.* 2022). Cancer immunotherapy might benefit greatly from the addition of IL-

12. These cytokines are released by both immunological and malignant cells. The tumor cell cycle is blocked, apoptosis is induced, and tumor cell growth is prevented by treatments focused on the IL-12 family of cytokines. Therapies that target IL-12 family cytokines and immune checkpoint inhibition are synergistic in nature (Deplanque *et al.* 2017, Eckert *et al.* 2017, Mills *et al.* 2019, Guo *et al.* 2017).

The correlation factor between IL-12 and other factors studied indicates that there is no significant difference . As shown as in Table 4.5 and Table 4.6

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

The category of children with an age of 1-10 years is more likely in brain cancer from other age groups.

The Lack of significant difference in urea in brain cancer and healthy patients.

The creatinine level was higher in brain tumor patients than in the healthy.

There's no significant difference in the level of alkaline phosphatase in patients than in healthy.

For CK-BB, it has been found that his level has decreased in patients than in the healthy.

For IL-12 , It has been found that his level has decreased in patients than in healthy .

5.2 Recommendations

Further studying the the extent to which biochemical factors in brain cancer patients are affected in periods of chemotherapy and radiation therapy

Further studying the creatine -kinase BB in cancer patients during the period of injury and treatment

Further studying the extent to which interleukin-12 is affected in brain cancer patients in radiation and chemical treatment periods.

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