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Article

Comparing The Alterations in Certain Electrolytes and Their Correlation with HIF-1α in Neuroblastoma Patients Pre- and Post-Chemotherapy.

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Abstract

Background: A significant obstacle to cancer treatment is the tumour microenvironment, namely tumour hypoxia. Hypoxia is a state in which the tumor's oxygen demand exceeds its supply, which is known to be linked to immunosuppression and decreased sensitivity to chemotherapy and radiation. Objectives: The aim of this study is to determine whether serum hypoxia-inducible factor-1 alpha, sodium, potassium, and chloride levels differ between children and adolescents with Neuroblastoma (NB) and controls, Also, monitor the effect of chemotherapy after three cycles on these parameters. Materials and methods: Three groups of 70 cases were created: Group A consisted of 35 healthy children who served as a control group; Group B comprised 35 newly diagnosed patients; and Group C consisted of the same newly diagnosed patients after three rounds of chemotherapy. The patients' ages ranged from two months to twelve years. From May 2022 to the end of March 2023, they were cared for in Basrah Specialist Hospital for Children. Results: The current study's results showed that patients with Neuroblastoma had decreased levels of the indicator hypoxia-inducible factor-1 alpha following three chemotherapy treatments, whereas

sodium, potassium, and chloride levels didn't change after three chemotherapy doses from when they were at the time of diagnosis. Conclusion: In order to enhance the prognosis of NB patients and create more effective and customised medicines that address NB heterogeneity, novel prognostic indicators and therapeutic targets are desperately needed in clinical settings.

Keywords: Neuroblastoma, chemotherapy, Hypoxia-inducible factor, sodium, potassium, and chloride.

Introduction

Cancers arise from the accumulation of oncogenic mutations[1]. Being relatively uncommon and originating in growing organs where cytotoxic medicines may have harmful effects, childhood tumours are challenging to study and cure[2], Table 1 displays southern Iraq's childhood cancer prevalence for the 2019–2021 timeframe. The most prevalent extracranial solid tumour that develops from the sympathetic nervous system's neural crest progenitor cells is called Neuroblastoma (NB) [3]. NB exhibits a wide range of clinical manifestations and results, partly because of variations in biologic and clinical prognostic characteristics[4]. It is responsible for 15% of paediatric cancer fatalities and 8% of paediatric cancer cases[5]. When NB is diagnosed, it has already spread to more than 50% of patients[6]. The most frequent genetic changes in neuroblastoma include DNA copy number changes, segmental chromosomal abnormalities, anaplastic lymphoma kinase (ALK) mutations, and MYCN amplification [7]. The most commonly impacted regions are the thoracic paraspinal part, the cervical region, the belly, and the adrenal glands. Because solid tumours retreat naturally and others are aggressive, these tumours may have different courses [3]. Children with solid tumours have three main options for treatment: chemotherapy, radiation therapy, and surgery. A localised, tiny, and non-metastatic tumour can benefit from surgery. If resection is not completed, there is a chance that the cancer will return. Even while they are effective, radiation and chemotherapy can have serious adverse effects and damage healthy cells [8]. In the majority of developing malignant tumours, the intratumor

microenvironment frequently exhibits hypoxia and increased HIF-1α expression. HIF-1α and hypoxia efficiently shield tumour cells from radiation, increasing their radio resistance. Similarly, enhanced chemoresistance has been associated with hypoxia and HIF-1α [9]. The first switch that promotes tumour blood vessel formation and development and extension is HIF-1α [10]. The physiological demands are considered to be the most fundamental need for human survival. Among these physiological requirements is the requirement for electrolytes and fluids, which come in second only to oxygen [11]. Many physiological processes in the body depend heavily on electrolytes. Normal organ function and many metabolic activities depend on precise intracellular and extracellular electrolyte concentrations [12]. This balance is essential for nerve impulses, muscular contraction, pH levels, and hydration. The main electrolytes are calcium, magnesium, sodium, potassium, and chloride. Significantly, the electrical characteristics of cardiac membranes are mostly determined by serum sodium, potassium, and chloride levels [13]. Electrolyte imbalances can make cancer patients' clinical condition and continued treatment more difficult. It is frequently possible to identify and treat the cause of these anomalies early on, improving the patient's adherence to chemotherapy. In order to provide efficient clinical care to cancer patients receiving treatment [14]. Our motivation for performing this study stems from the high incidence of childhood cancer in southern Iraq, particularly in the Basra Governorate, which is caused by environmental pollution from burning oil and other pollutants, as well as war residuals. Additionally, this study was carried out to determine the potential alterations the tumour may produce in the body and the impact of chemotherapy following three treatment cycles on the HIF-1α, Na, K, and Cl levels because there aren't many studies that examine solid tumours, particularly neuroblastoma.

Table 1: Cancer in children in southern Iraq 2019–2021 (Basra Health Department / Iraq, 2022).

Childhood Cancer	Percentage		
	2019	2020	2021
Bone tumor	0.8 %	1.05 %	1.39 %
Muscle tissue cancer	0.414 %	0.79 %	0.695 %
Leukemia	7.63 %	3.52 %	8.36 %
Lymphoma	1.26 %	1.84 %	1.989 %
Brain tumor	0.887 %	1.9 %	0.26 %
Neuroblastoma	1.67 %	1.65 %	1.3 %
Kidney cancer	1.08 %	1.27 %	1.05 %
Retinal tumor	0.26 %	0.39 %	0.48 %
Tumor of the nose and pharynx	0.039 %	0.11 %	0.047 %
histiocytic tumour	0.079 %	0.225 %	0.22 %
Fetal tissue tumor	0.157 %	0.11 %	0.22 %
Soft tissue tumor	0.177 %	0.17 %	0.096 %
lung tumor	0.039 %	0.02 %	0.048 %

Materials and Methods

From May 2022 until the end of March 2023, 70 research participants were enrolled in the current study. Three groups of 70 children—boys and girls—were formed from the participants. At the Al-Basra Children's Teaching Speciality Hospital in the southern Iraqi province of Basra, they were divided into three groups: group A consisted of 35 healthy children as a control group; group B consisted of 35 newly diagnosed patients; and group C consisted of the (same patients prior to chemotherapy) after three cycles of treatment the ninth week. They were between two months to twelve years old. Doctors were able to diagnose patients clinically by performing specific standard blood tests on them. Parents of children with illnesses provided written informed consent.

"Blood samples totaling 6 mL, were taken from both the controls and the patient. Samples were centrifuged at 3000 x g for 10 minutes after allowing to coagulate at room temperature in empty disposable tubes if not used right away.

Serum samples, were separated and kept at (-20 ° C), for later assessment of biochemical markers". The serum was utilized to estimate the levels of HIF-1α using an ELISA (Enzyme-Linked Immunosorbent Assay). Using flame atomic absorption photometry, serum electrolytes such as sodium, potassium, and chloride were measured.

Statistical Analysis

"The statistical analysis results are presented as mean and standard deviation (mean ± SD) using SPSS version 23. To compare the subgroups, a one-way ANOVA was utilized. Pearson's correlation revealed that the parameters of the current investigation were connected. P-values (less than 0.05) have been used to evaluate statistical significance".

Result

The concentration of HIF-1α in the B group was significantly higher than in the A and C groups ($p \leq 0.05$), as indicated by Table (2) and figure (1) The concentration of HIF-1α was found to be significantly higher in the C group than in the A group ($p \leq 0.05$).

Table 2: shows the patient and control groups' serum HIF-1α levels.

Groups	No.	HIF-1α (ng/dL) (Mean ±SD)
A	35	26.01 ± 4.76 [c]
B	35	57.60 ± 7.47 [a]
C	35	48.25 ± 9.16 [b]
LSD		2.92

"A: control, B: Before chemotherapy , C: after chemotherapy. SD represents the Standard deviation. No represents the Number of subjects. LSD represents the Least Significant Difference (a, b, c) indicates having various letters in same column have been significantly differed (P <0.050)".

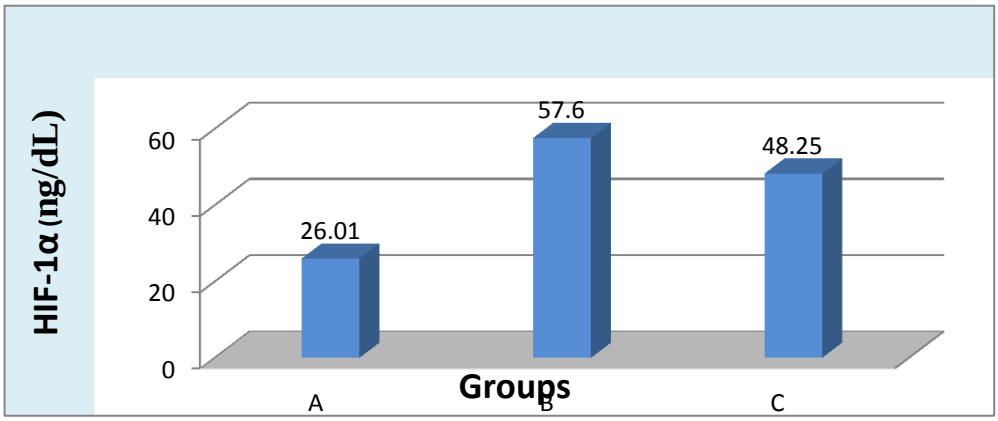

Figure 1: shows the serum HIF-1α levels in the patient and control groups.

All research groups' Na concentrations do not differ significantly (p≤0.05), according to Table (3) and figure (2). Also, all research groups' K concentrations do not differ significantly (p≤0.05), according to the same Table (3) and figure (2). Additionally, there is no discernible difference in CL concentrations across all research groups (p≤0.05) according to the same Table (3) and figure (2).

Table 3: shows the patient and control groups' serum Na, K and Cl levels.

Groups	No.	Na (mmol/L) (Mean ± SD)	K (mmol/L) (Mean ± SD)	CL (mmol/L) (Mean ± SD)
A	35	142.94±5.66 [a]	3.90±0.40 [a]	100.86± 5.94 [a]
B	35	142.71±5.18 [a]	3.97± 0.43 [a]	101.29± 5.82 [a]
C	35	141.26± 5.10 [a]	4.09± 0.45 [a]	101.60± 5.91 [a]
LSD		2.11	0.27	2.33

Legend as in Table 2.

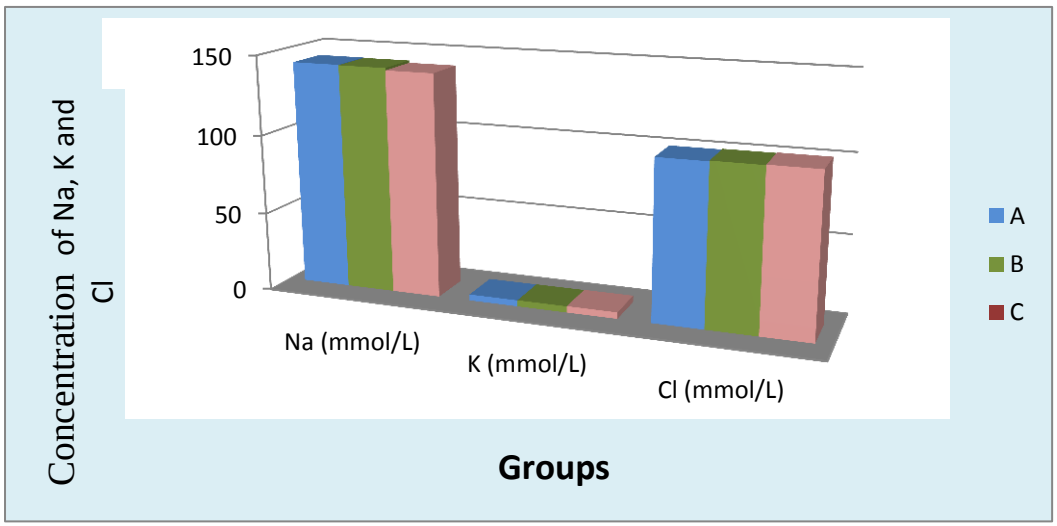

Figure 2: shows the serum HIF-1α levels in the patient and control groups.

Figure 3 indicates that HIF-1 α and Na levels are positively correlated both before and after chemotherapy (r = 0.06 and 0.14 respectively).

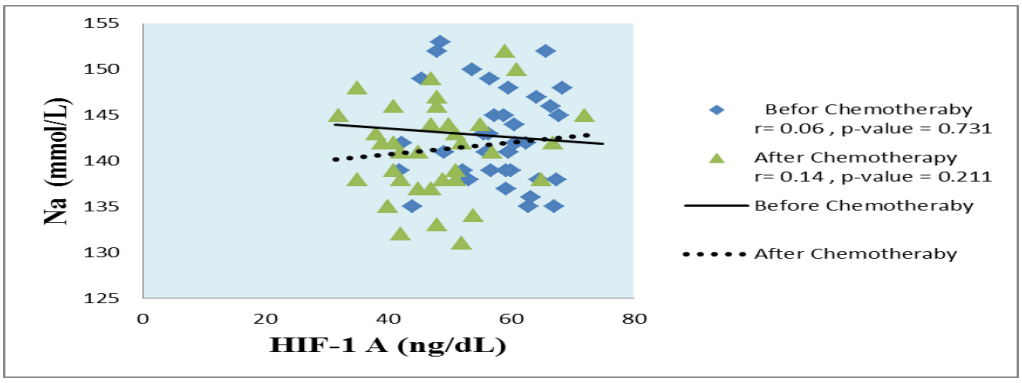

Figure 3: Serum HIF-1α and Na correlation in patient groups.

Figure 4 indicates that HIF-1 α and K levels are positively correlated both before and after chemotherapy (r = 0.03 and 0.04 respectively).

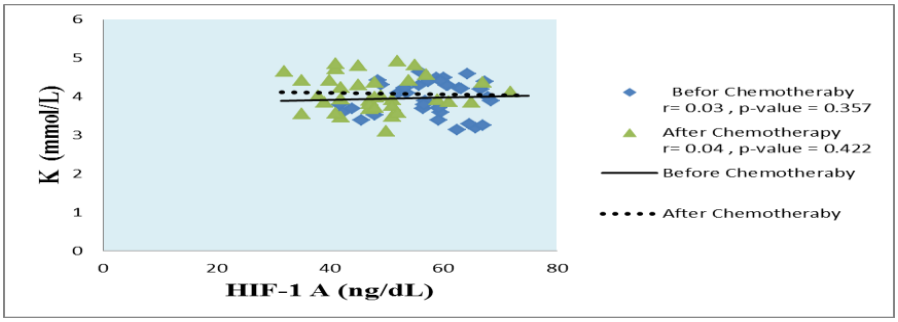

Figure 4: Serum HIF-1α and K correlation in patient groups.

Figure 5 indicates that HIF-1 α and K levels are positively correlated both before and after chemotherapy (r = 0.02 and 0.02 respectively).

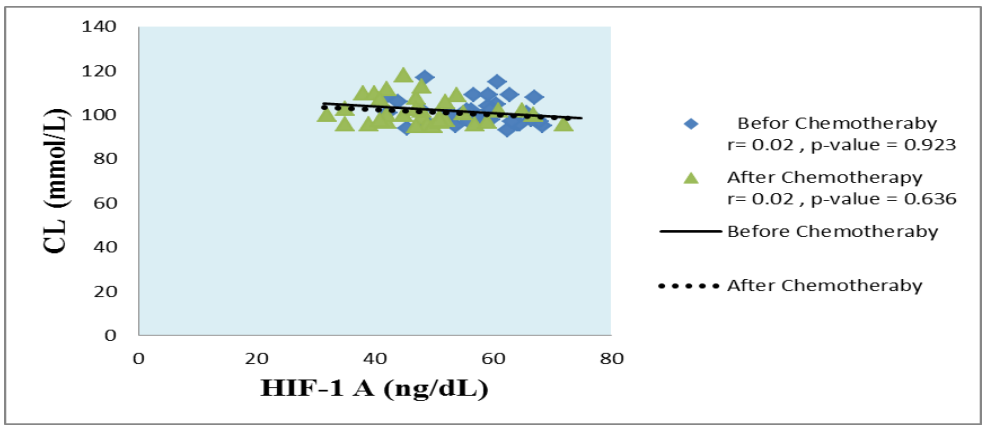

Figure 5: Serum HIF-1α and Cl correlation in patient groups.

Discussion

The cellular processes that propel the growth of cancer are extremely complex and comprise a network of interrelated events. These include intricate signalling networks that support the growth and survival of cancer cells as well as genetic abnormalities that start the process of tumorigenesis[15]. Numerous solid cancer forms experience hypoxia, which is the decreased availability of oxygen relative to the tissues' need for it [16]. One of the main regulators of the tumour microenvironment (TME) and a factor in controlling cancer's hallmarks is hypoxia [17]. Several studies have demonstrated that the TME has a significant impact on the ability of malignant cells to proliferate and spread. Evidence suggests that immune effector gene expression in T and NK cells may be suppressed by hypoxia in the TME, leading to immune cell malfunction and immunotherapy resistance[18]. Chemotherapy medications kill cancer cells, but they can also kill healthy cells. Most chemotherapy side effects are transient. However, there may be some long-term consequences [19]. Two groups were formed from the participants according to the length of therapy. In this study, serum level of each parameter was investigated (HIF-1α, K , Cl and Na) before and after three cycles of chemotherapy treatment. Depending on the patient's reaction to treatment, chemotherapy is often administered in two to eight cycles.

Chemotherapy pulses occur at intervals of at least three weeks. All of the participants in our study were in stage IV. In the current investigation, patients in the NB group were monitored in accordance with the treatment protocol following three chemotherapy doses (following the ninth week of treatment) in order to evaluate their physiological and biochemical reactions. Chemotherapy medications, including doxorubicin (adriamycin), vincristine, cyclophosphamide, etoposide, and cisplatinum, were routinely administered to children of NB patients during the induction phase.

The surrounding tissue provides the nourishment and oxygen that tumours need to survive. When a tumour becomes big enough, it needs its own blood supply to keep up with its growing metabolic demands [20]. According to our findings, hypoxia is a characteristic of NB and a factor in the poor prognosis. As the current investigation found that NB had significantly higher serum HIF-1α levels prior to chemotherapy than did healthy participants, suggesting that this parameter is an indication of malignancy. Tumour tissues with high HIF1α protein expression may have undergone tissue necrosis, which led to a significant amount of HIF-1α entering the bloodstream [21]. Tissue survival in a hypoxic environment can be improved and anaerobic metabolism of tumour tissue further encouraged by elevated HIF-1α expression [22].

Since hypoxia is generally linked to chemotherapy and radiation resistance, we examined patients' HIF-1a levels after the third round of treatment and found that they had dropped. When chemotherapy causes more neoplastic cells to die, it can reduce the size of lesions and improve vascularization in cancer cells because tumours have irregular vascularization and O2 cellular imbalance. Therefore, decreased HIF-1α levels after the start of chemotherapy may be useful for predicting the efficacy of chemotherapy. Chemotherapy increased malignant tissues' oxygenation, which in turn decreased HIF-1α expression [23].

One factor contributing to the improved prognosis of children with cancer is the use of protocols that include many cytostatic medications; nonetheless, damage to diverse organs is possible. The kidney is a target that is especially

vulnerable to medication metabolism and clearance [24]. Electrolyte imbalances can cause symptoms that might impair quality of life and interfere with some chemotherapy treatments. Corrective therapy and early diagnosis of these conditions are therefore essential to the treatment of cancer patients [25]. The fact that there were no appreciable differences in serum levels of Na, K, or Cl among the three groups under investigation in this study suggests that chemotherapy is not directly tubulotoxic. Typically, its glomerular side effects have no clinical significance [26]. Even though the current study did not find any clinical side effects, it is important to consider the kidney risk, which might manifest during or soon after treatment, days or weeks later, or months or even years after chemotherapy is finished.

Conclusion

The results showed that NB patients who had three doses of chemotherapy had reduced levels of the HIF-1α parameter, but their levels of Na, K, and Cl remained constant before and after treatment. As a result, appropriate patient follow-up is essential. For paediatric patients, it is crucial to keep in mind that the sample size is too small to draw firm conclusions. Further prospective investigations are essential to bolster the findings of this study.

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