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The Hemotoxicity of Chemotherapeutic Regimens in Sudanese Children with Retinoblastoma

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Abstract

Background: There has been a rapid increase in cancer among Sudanese citizens from 1999 until this year. At least 80% of all patients who undergo chemotherapy will develop anemia as a complication. This inpatient analytical comparative study aims to examine the possible association between hemotoxicity and various chemotherapy regimens in Sudanese children diagnosed with retinoblastoma.

Methods: This study enrolled 30 patients diagnosed with childhood retinoblastoma who were admitted from June 2006 to September 2008 to the Radiation and Isotope Center Khartoum. We collected 90 blood samples to examine for a possible association between anemia and the chemotherapeutic regimen. All patients (n=30) were included in each arm of the chemotherapy regimen.

Results: Prior to the onset of chemotherapy, 50% of patients had normal hemoglobin levels, 43.3% had mild anemia, and 6.7% had moderate anemia. Post-cycle I treatment, there were only 6.7% who had normal hemoglobin levels. Mild anemia was observed in 60%, followed by 30% for moderate anemia and 3.3% of patients had severe anemia. Post-cycle II there were no patients with normal hemoglobin levels, however 26.7% had mild anemia and the majority of patients (approximately 73.3%) had moderate anemia.

Conclusion: A correlation existed between hemoglobin values after completion of therapy to the overall treatment. We observed a decline of 1 to 2 g/dl in hemoglobin levels.

Keywords: Cancer, Solid tumor, Retinoblastoma, Chemotherapy, Anemia

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Introduction

Retinoblastoma is a relatively uncommon tumor of childhood that arises in the retina and accounts for about 3% of the cancers in children younger than the age of 15 years.[1,2] The prognosis for a patient with recurrent or progressive retinoblastoma depends on the site and extent of the recurrence or progression.[3,4]

According to the Federal Ministry of Health of the Republic of Sudan, the incidence of cancer in Sudan has been rapidly increasing from 1999 until this year. At least 80% of all patients who undergo chemotherapy develop anemia as a complication of this treatment.[5] The availability of recombinant human erythropoietin (rHuEPO) has opened up a number of therapeutic options. The earliest licensed clinical indication for this medication was for the management of transfusion-dependent anemia in patients with renal failure who were undergoing hemodialysis.[6, 7]

This study aims to classify chemotherapy-induced anemia related to childhood cancer according to the severity of anemia. In order to improve patient quality of life (QoL) and minimize the need for blood transfusions, the study results aim to assist oncologists with new insights and introduce rHuEPO as a therapeutic treatment. This study also assesses the presence of hemotoxicity during various chemotherapy regimens.

Materials and Methods

An inpatient analytical comparative study was conducted to examine the possible association between anemia, chemotherapeutic regimen, duration and stage of childhood retinoblastoma. The study was performed from June 2006 until September 2008 in the major specialized medical Radiation and Isotope Center Khartoum (RICK).

Inclusion criteria

Included were infants and children aged 6 months to 15 years who were diagnosed with retinoblastoma and planned to receive chemotherapy. The study groups had the following hemoglobin (Hb) levels: normal (≤10.9 g/dl), mild (9.5-10.9g/dl), or moderate (9.0-9.4 g/dl) prior to the initiation of the therapeutic regimen.

Exclusion criteria

Exclusion criteria included any anemia other than malignancy-related, such as nutrition (iron, B12 or folate deficiencies) and occult blood loss from hemolysis and bleeding. Also excluded were patients with Hb levels below 9.0g/dl prior to the initiation of chemotherapy whose anemia were associated with the underlying disease and not from other therapeutic regimens such as radiotherapy, hormone therapy and immunotherapy. Patients with normal Hb levels who were transfused with whole blood or packed red cells within three weeks of study entry were also excluded.

Sampling technique and size

In view of the design of this study, we selected cases by a non-probability technique; in particular, we performed quota sampling in which the study population was divided into categories and a quota was surveyed from each category. Since non-probability samples did not involve any mathematical rules, only 30 cases were followed and investigated. All study groups (n=30) were included in each arm of the chemotherapy regimen. Each case had three blood samples drawn as follows: sample one was obtained before starting the therapeutic regimen (pre-cycle I). Sample two was obtained post-cycle I of the induction phase of chemotherapy (three weeks after the first chemotherapy dose); and sample three was taken post-cycle II in the induction phase of chemotherapy (three weeks after the chemotherapy dose). There were 90 blood samples analyzed by medical laboratory techniques, using a simple automated complete blood count (CBC) technique. Patient samples were obtained after the diagnosis of retinoblastoma.

Collection technique

The Study was approved by the local Ethics Committee in Radiation and Isotope Center Khartoum (RICK). Consent was obtained from patients' parents after they were informed about the research objectives, its importance in therapy and follow up. Blood samples were collected by phlebotomy vein puncture in a procedure room. Blood samples were collected in the early morning hours, between 8-11 am, pretreatment samples, three weeks after the first dose (post-cycle I) of chemotherapy and three weeks post-cycle II of chemotherapy. Samples were analyzed by an automated hematology analyzer, model KX-21N, which performs blood cell counts by the direct current (DC) detection method.[8]

Statistical analyses

The independent sample t-test was used to determine significance differences to obtain the probability values (<0.05 *P* Value setting as significance variations).

Results

Between June 2006 and September 2008 we evaluated 30 infants and children who were divided into two age groups, less than 2 years and those over the age of 3 years. Patients were diagnosed with retinoblastoma (intraocular, extraocular or recurrent) and all planned to receive chemotherapy. Most (66.6%) had extraocular disease (Table 1).

Hemoglobin levels in the pre-cycle I (control) and after two cycles of chemotherapy (post-cycles I and II) were determined during various chemotherapy regimens. A normal Hb level of 11.0 g/dl in pre-cycle I was observed in 2 (6.7%) patients and 8 (26.7%) patients in post-cycles I and II. For the Hb levels of 8-10 g/dl, various abnormalities were observed in post-cycles I and II compared to normal values seen in pre-cycle I (Table 2). Table 3 depicts the hematologic characteristics of the study patient population in pre-cycle I, and post-cycles I and II.There were

Table 1. Patients' characteristics.

Variables	Patients (n)	%
Total patients	30	
Female	16	53.3
Male	14	47.7
Age (years)		
<2	10	13.3
2 – 3	5	16.7
>3	15	50.0
Duration(years)		
<0.5	14	46.7
0.5 – 1	13	43.3
>1	3	10
Stages		20
Intraocular	5	16.7
Extraocular	20	66.6
Recurrent	5	16.7

changes in treatment schedules related to some hematological parameters of total white blood cell (TWBC), Hb, red blood cells (RBC), platelet count and absolute neutrophil count (ANC).Table 4 lists the overall hematological changes attributed to the chemotherapy regimens compared to the pre-cycle control. In the group of patients who received vincristin (VCR) and dactinomycin (ACTD), the mean levels of TWBC and ANC significantly decreased in post-cycle I, whereas Hb, RBC, and platelet counts did not differ significantly.

In patients who received VCR and doxorubicin (DOX), the mean levels of TWBC, Hb and platelet count significantly decreased in post-cycle I and post-cycle II. A significant decrease in ANC was observed in post-cycle II regimens. The mean RBCs and ANC were significantly similar to the platelet countsin post-cycle II regimens.Vincristin, ACTD and cyclophosphamide (CPM) regimens that were administered to stage III tumor patients significantly differed in terms of mean TWBCs, Hb, platelet count and ANC. However RBCs did not significantly differ in post-cycle I. In post-cycle II there was a significance difference in TWBCs, Hb, platelet count, RBCs and ANC values. Patients who received cisplatin (CIS) and DOX showed significant decreases in their

Table 2. Distribution of hemoglobin (Hb) levels among children with retinoblastoma.

Mean Hb g/dL(%)	Pre-cycle I (control) n (%)	Post-cycle I n (%)	Post-cycle II n (%)
11.00 (73.3)	15 (50)	2 (6.7)	8 (26.7)
10.00 (66.6)	13 (43.3)	18 (60)	22 (73.3)
09.00 (60.0)	2 (6.7)	9 (30)	0 (0.0)
08.00 (53.0)	0 (0.0)	1 (3.3)	0 (0.0)
Total	30 (100)	30 (100)	30 (100)

mean TWBC, Hb, and the ANC, whereas there were no significant decreases observed for RBC and platelet count in post-cycle I. In the CIS, VP-16 and adriamycin (ADR) with CPM regimens, TWBC, Hb, and ANC significantly decreased, but there was no significant difference in RBC and platelet count in post-cycle I. In post-cycle II the TWBC, Hb, RBC, platelet count and ANC significantly decreased. Patients who received VCR and carboplatin (CAR) regimens had significantly decreased TWBC, Hb, and ANC values, whereas RBC and platelet count did not significantly differ in post-cycle I. In post-cycle II, TWBC, Hb, RBC, platelet count and ANC significantly decreased. Patients who received ifosfamide (IFS) and etoposide (VP-16) in stage VII disease only had significantly decreased mean TWBC, Hb, RBC, and ANC values compared to the control, whereas the platelet count did not significantly differ compared to the control (Table 4).

Discussion

Retinoblastoma, the most common intraocular tumor in children, is a rare disease with an incidence of 1:17 000 live births. It is inherited as an autosomal dominant trait.[10] In numerous trials that have been conducted in adult cancer patients, treatment with recombinant erythropoietin (EPO) has been shown to increase Hb levels, reduce RBC transfusion requirements and improve QoL.[11] Much less has been published of its use in the prevention or treatment of cancer-associated anemia (CAA) in children, in whom chemotherapy is usually more intense and likely to result in greater myelosuppression. Laboratory results and general clinical observations during two cycles of induction phase chemotherapy have shown low Hb levels before patients presented with severe anemia symptoms such as pallor, tachycardia, anorexia and dysponia. These results were observed in children who initiated chemotherapy with Hb levels above or equal to 9.0 g/dl, prior to the need for blood transfusions.[12] Although anemia and its impact on patient's social function and QoL has been the main goal of this study, there were other hemotoxicities observed. Of these, the most important were the spontaneous declines in WBC and ANC levels. Both WBC and ANC have been shown to play important roles in the immune system defense mechanism. The third hemotoxicity observed was a decline in platelet count. Evaluation of platelet count post-treatment is important for the prevention of thrombosis or hemorrhage. Thus, the Department of Pediatric Oncology at RICK have established guidelines for treatment of toxicities.[13] Most patients treated for retinoblastoma in developing countries become long-term survivors. It is well known that the disease develops in an asymmetrical pattern. At diagnosis, usually one eye is more severely affected than the other.[14]

Treatment doses were delivered by intravenous infusions (i.v.) in conjunction with hydration by D5 normal saline and magnesium sulfate ($MgSO_4$) in order to reduce the nephrotoxicity and ototoxicity of CIS. Dexamethasone tablets were administrated to reduce swelling. Cycle I treatment schedules

Table 3. Hematological parameters in control (pre-cycle I) and post-cycles I and II chemotherapy among children with retinoblastoma.

	Post-cycle I			Post-cycle II		
	Control Mean	Patients Mean	*P*-value	Control Mean	Patients Mean	*P*-value
TWBCs (c/L)	8.05	6.45	0.007	8.05	5.64	0.000
Hb (%)	73.30	65.10	0.000	73.30	57.93	0.000
PCV (%)	33.73	30.00	0.000	33.73	27.76	0.000
RBCs (c/L)	4.53	4.04	0.001	4.53	3.71	0.000
MCV (fl)	74.15	74.14	0.997	74.15	72.18	0.443
MCH (pg)	26.64	24.13	0.005	26.64	22.65	0.000
MCHC (%)	32.12	29.96	0.002	32.12	27.63	0.000
Plt (c/L)	438.86	421.50	0.620	438.86	383.86	0.125
Neut (%)	51.63	44.53	0.074	51.63	41.00	0.014
Lymph (%)	40.70	50.10	0.024	40.70	52.40	0.007
Mono (%)	4.67	4.20	0.637	4.67	4.90	0.827
Eosin (%)	1.70	1.10	0.181	1.70	1.53	0.751
Baso (%)	0.13	0.20	0.628	0.13	0.266	0.498
ANC	4075.46	2895.43	0.005	4075.46	2226.10	0.000

TWBC: Total white blood cell; MCH: Mean cell hemoglobin; Hb:Hemoglobin; MCHC: Mean cell hemoglobin;PCV: Packed cell volume; Plt: Platelet count;RBC:Red blood cell; Neut: Neutrophils; MCV: Mean cell volume; Lymph: Lymphocytes; Eosin: Eosinophils; Mono: Monocytes; Baso: Basophils; ANC: Absolute neutrophil count

included VCR, ACTD, DOX, CPM, CIS, VP-16, ADR and CAR. Cycle II treatment schedules included VCR, DOX, IFS, CIS, VP-16, ADR, CPM and CAR. Between treatments, there was a 21-day rest period, the length of which was dependent on hematological recovery as evidenced by an ANC level ≥1000 and a platelet count ≥100×10^9/L. The treatment protocol varied according to the disease and its stage.[15]

The lowest mean value for leucopenia was observed.[16] The highest mean value observed was 7.32 in cycle II of the VCR and Dox regimen.

Anemia, defined as a decline in Hb concentration and reduced red cell count,[17] was observed as follows. There was a highly significant decline observed in post-cycle I (*P*=0. 000) which was similar to post-cycle II (*P*=0. 000) although the mean difference compared to the prior administration in post-cycle I was 15.3 while the mean difference compared to the prior administered dose in post-cycle II was 8.1.

Thrombocytopenia due to a decline in platelet count to below normal limits (<150×10^9/L)[18] was absent in the two cycles post-treatment, since most patients began treatment with an upper limit of normality to an increased platelet count (>450×10^9/L) which correlated statistically, as seen in post-cycle I (*P*=0. 62) compared with the post-cycle II (*P*=0. 125). The increase in platelet counts in this retinoblastoma was associated with an inflammatory response in addition to the strong relation between EPO and thrombopoietin (TPO). TPO is a cloned growth factor for megakaryocyte maturation and platelet production. The TPO protein is larger than EPO but half the protein structure has an identity with or similarity to EPO at the N-terminal region.[19] The exact role of a TPO on erythropoiesis is not known but it is well recognized that several types of anemia from chronic diseases are the result of TPO. These observations highlight the close relationship between red blood cell and platelet production control.[4]

The treatment regimens in retinoblastoma

Table 4. Chemotherapy schedule and hematological changes in pre-cycle I, post-cycles I and II.

Regimen	TWBCs(c/L) Mean (*P*-value)	Hb (%) Mean (*P*-value)	RBCs (c/L) Mean (*P*-value)	Plt (c/L) Mean (*P*-value)	ANC Mean (*P*-value)
Pre-cycle I	8.05	73.30	4.53	438.86	4075.46
Post-cycle I					
I	5.97(0.000)	72.67(0.130)	4.15(0.120)	464.67(0.210)	2492.00(0.000)
II	7.32(0.000)	62.67(0.000)	4.50(0.430)	511.67(0.050)	4330.83(0.124)
III	4.70(0.000)	68.00(0.000)	4.20(0.130)	609.00(0.000)	7668.00(0.000)
IV	6.50(0.030)	61.50(0.000)	4.10(0.120)	430.00(0.321)	2605.00(0.000)
V	7.27(0.050)	57.20(0.000)	3.85(0.070)	360.33(0.130)	2583.83(0.000)
VI	6.42(0.040)	65.83(0.000)	4.00(0.060)	343.00(0.120)	2611.50(0.000)
Post-cycle II					
II	6.52(0.040)	65.67(0.050)	3.84(0.030)	427.00(0.211)	2655.33(0.000)
III	4.62(0.000)	55.50(0.000)	3.61(0.020)	398.33(0.030)	2163.17(0.000)
V	6.02(0.030)	55.00(0.000)	3.52(0.030)	299.17(0.020)	1780.83(0.000)
VI	5.55(0.000)	57.83(0.000)	3.70(0.050)	326.67(0.030)	1903.83(0.000)
VII	5.50(0.000)	55.67(0.000)	3.61(0.020)	398.33(0.060)	2627.33(0.000)

I: VCR+ACTD (VCR: Vincristin, ACTD: Dactinomycin); II:VCR+DOX (DOX: Doxorubicin); III:VCR+ACTD+CPM (CPM: Cyclophosphamide); IV:CIS+DOX (CIS: Cisplatin); V: CIS+VP-16+ADR+CPM (VP-16: Etoposide, ADR: Adrymicin); VI: VCR+CAR (CAR: Carboplatin); VII: IFS+VP-16 (IFS:Ifosfamide); TWBC: Total white blood cells; Hb: Hemoglobin; RBC: Red blood cells; PLT: Platelets; ANC: Absolute neutrophil count

patients showed less severe hematological toxicity; these results agreed to a study by Abramson.[14] However severe toxicity was observed in stage IIB retinoblastoma.

The treatment regimens according to the stage were as follows: stages I, IIA, IIB and III received courses of VCR ACTD and CPM in both cycles. Stages IV and IVS patients received CIS and DOX in the first cycle followed by VCR, ACTD, and CPM in cycle II.

Hematologic toxicities according to treatment cycle were as follows. Prior to treatment (pre-cycle) there were 10% of patients who were normal and 10% who had moderate anemia. Post-cycle I treatment resulted in 13.3% of patients with mild anemia, while 6.7% had moderate anemia. Post-cycle II treatment showed 3.3% of patients with mild anemia, but 16.7% had moderate anemia. The results agreed with the expected results according to the WHO criteria which was based on cancer type and the appropriate protocol.[9]

Blood morphology was examined as thin blood films stained with Leishman stain. Patient's pretreatment treated blood morphology showed a normocytic normochromic red cell morphology with normal white blood cell distribution and morphology, and platelets that were either normal in morphology or giant sized with few aggregations. Post-treatment cycles I and II showed the same morphological appearance, which was an isopoikilocytosis with few target cells. White blood cells and platelets were normal in morphology. The RBC morphological changes could have been attributed to either the effect of chemotherapy on the bone marrow, although white blood cells and platelets retained their normal morphologies, or a change in the primary site of hemopoiesis, which was accompanied by simultaneous changes in morphology.[20]

There was no family history of any cancer type reported, particularly among first-degree relatives and no association was observed between chemotherapy dose and anemia. Another problem was that comparison between this study's results

and other childhood solid tumor studies was not found. In the two cycles of induction phase chemotherapy studied, there were no requirements for any RBC or platelet transfusions or the need for granulocyte CSF.

In conclusion, among petreatment patients the normal blood levels of anemia were seen in 50% of patients, followed by 43.3% with mild anemia and 6.7% who had moderate anemia. Post-cycle I treatment showed a decline in normal levels to 6.7% of patients, whereas the groups with mild anemia comprised the majority (60%) followed by 30% of patients with moderate anemia and 3.3% that had severe anemia. At post-cycle II, there were no patients that had normal levels, however mild anemia was observed in 26.7%. The majority of patient's (73.3%) had evidence of moderate anemia. A correlation between Hb values after completion of therapy to overall treatment was found as a decline in the range of 1 to 2 g/dl compared to after treatment.

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