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The Role of Regulatory T cell in acute leukemia and associated febrile infections

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Abstract

Background: Regulatory T (Treg) cells control peripheral immunetolerance. Patients with cancer, including those with hematologic malignancies, have elevated numbers of Treg in the peripheral circulation and in tumor tissues. However, mechanisms of suppression and clinical significance of Tregin acute leukemia have not been well defined. **Objective:** Investigate T reg cells frequency in patients with acute leukemia before and after treatment, and determine how they are affected by febrile infections. **Methods:** T reg in the peripheral blood of twenty five leukemic patients (before and after remission) and twenty age-matched healthy controls were counted by flow cytometry. Correlations between the frequency of circulating T reg cells and treatment outcome and infection were evaluated. **Results:** There was significant increase in percentage (%) of CD4+ CD25+ T lymphocytes to total lymphocytes among leukemic patients after induction in comparison to before induction.Leukemic patients after induction had a statistically significant decrease in absolute numbers of CD4+CD25+ in comparison to control and patients before induction. The absolute number of CD4+CD25- in the cases after induction was significantly lower in comparison to before induction and control. Patients during fever showed a statistically significant increase in % of CD4+CD25+ in comparison to before induction. **Conclusion:** The frequency of T reg cells in peripheral blood may be used as a biomarker for predicting sensitivity to chemotherapy and prognosis of acute leukemia. Additionally, manipulation of Treg cells can modulatethe immune systemand represent new strategies for cancer treatment.

Keywords: regulatory T cells, acute leukemia, febrile infections, CD4+CD25+.

Introduction

Leukemia isgroup of cancers originating from blood-forming tissues. In this disorder, the bone marrow produce an abnormally large number of immature white blood cells (WBCs). These leukemic WBCs eventually replace the normal ones, resulting in the clinical manifestations of anemia, bleeding tendency and infection (Bennett et al., 1976). Leukemia is classified into four main subtypes according to cell type (lymphocytic/myeloid) and rate of growth (acute/chronic). Acute leukemia includes acute lymphocytic leukemia (ALL) derived from immature T or Blymphocytes and acute myeloid leukemia (AML) derived from immature myeloid cells(Bray et al., 2012). Leukemia is one of the most common malignancies in Jordan, Lebanon, Bahrain, Egypt, Iraq, Libya, Kuwait, Oman, Qatar, Saudi Arabia,

Syria, and the UAE. The two most common types of leukemia in children and adolescents are ALL followed by AML in both Arab countries (Tadmouri et al., 2015) and the US (American Cancer Society, 2014). ALL accounts for 80% of leukemic cases in children and 56% in adolescents. AML is less common. Leukemia associated mortality accounts for 0.1% in Egypt, and is categorized as the 3rd most common mortality-causing neoplasm. It remains the second leading cause of death in children aged 5-14, although advances in treatment have increased the survival rate(Elgendi et al., 2010; Bray et al.,2012).
Naturally occurring tumor immunity involves cellular components of both the innate and adaptive immune system. Several T cell subpopulations are involved in these immune responses (Sakaguchi, 2004).One of these are regulatory T (Treg) cells, known to be CD4+CD25+. Treg cells represent 1–10 % of total CD4+ T cells in the thymus, peripheral blood and lymphoid tissues. They can inhibit the function of effector T cells in both a contact dependent and cytokine independent manner (Schmidt et al., 2012).Treg cells may also play an important role in immune evasion mechanisms employed by human cancer. However, the exact mechanism of action of Treg remains unknown(Tokuno et al., 2009).

Treg cells contribute to the maintenance of self-tolerance. They can suppress the function of auto-reactive cells that escape thymic negative selection (Fehervari and Sakaguchi, 2004).The observed lack of responses to immunotherapy in cancer patients may be contributed to a crucial mechanism of tumor evasion mediated by Treg immunosuppression (Gattinoni et al., 2006). Mechanisms of Treg-mediated suppression of antitumor immunity are under investigation. Various studies have identified several distinct mechanisms for elimination of responder Tcells, including activation of perforin dependent or granzyme B-dependent pathways, production of interleukin (IL)-10 and transforming growth factor beta (TGF)-β1 by Treg, or up-regulation by Treg of indoleamine-2,3-dioxygenase expression in antigen-presenting cells. Adenosine triphosphate hydrolysis has also emerged as another Treg-mediated mechanism of suppression (Beyer and Schultze, 2006; Zou, 2006).

Increased population of Treg has been observed in patients with ovarian cancer, breast cancer, gastrointestinal cancer and lymphoma (Woo et al., 2001; Liyanageet al., 2002; Marshall et al., 2004; Beyer and Schultze, 2006; Strauss et al., 2007b).

Furthermore, studies have revealed that the frequency of CD4+CD25+ Treg was increased in acute leukemia (Wang et al., 2005; Miroslaw et al., 2009; Szczepanski et al., 2009; Ustun et al., 2011; YangandXu,2013)and the expression of CD25high has been used for definition of the human Treg subset (Dieckmann et al., 2001; Jonuleit et al., 2001).The accumulation of Treg and their increased suppressor function in cancer patients with advanced disease suggest that immune suppression is linked to tumor progression. Indeed, the increased proportion of Treg in the peripheral blood or at the tumor site has been reported to correlate with a poor disease outcome (Curiel et al., 2004; Griffiths et al., 2007).

Tumor cells are a rich source of TGF-β. Cytokines produced from tumor cells as TGF- β and IL-10, cause recruitment of Treg to the tumor site directly or indirectly through conversion of CD4+CD25-T cells into CD4+CD25+ Treg cells. This mechanism enables the tumor cells in evading the immune system, so that converted Treg, will suppress the anti-tumor immunity (Liu et al., 2007). That indicates that depletion of Treg could reverse that suppression and therefore, proliferation of effector T cells and tumor rejection. Antony et al. reported that selective elimination of CD4+CD25+ T cells in mice results in tumor growth inhibition or, in some instances, tumor regression. However, adoptive transfer of CD4+ CD25+ T cells is associated with suppression of antitumor immunity and tumor progression (Antony et al., 2005). Elimination of Treg cells would confer a greater therapeutic potential in cancer immunotherapy. It permits CD8+T cell-mediated suppression against tumors and favors memory T cell against tumors (Casares et al., 2003).This can be mediated by monoclonal antibodies directed towards the host immune cells that act as inhibitory checkpoints on the immune system as antibodies directed to CD4+CD25+Treg (Hodi et al., 2003).

Among the most common symptoms in leukemic patients are infections. Fever usually accompanies neutropenia in hematological malignancy and may be the first and only sign of sepsis. This risk of infection continues to grow as the intensity and duration of chemotherapy extends (Freifeld et al., 2011). Several mechanisms by which leukemic cells evade normal immune monitoring have been suggested, including alterations in T lymphocyte apoptosis, secretion of cytokines and expression of adhesion or signaling molecules in T cells (Sharmaand Settleman, 2007).

Decrease in the number of Treg has been found in the peripheral blood of patients with chronic infections. This decrease may bedue to redistribution of Treg rather than an overall decrease, as Treg accumulate at sites of the disease (Anderson et al., 2005).Therefore the aim of this study is to determine disturbances in Treg cells frequency in patients with acute leukemia before and after treatment, and to determine the effect of febrile infections on Treg in such patients.

Subjects and Methods

This prospective study was conducted on twenty five patients diagnosed on the basis of clinical findings and investigations as leukemia (ALL and AML). They were selected from the Department of Hematology Ain Shams University Hospital between August 2013 and May 2014. Twenty apparently healthy individuals matched for age and sex were included in this study as controls. An informed consent was obtained from all participants, and the study was approved by the Research Ethics Committee of Faculty of medicine-Ain Shams University.

Study design:

Patients were divided in two groups; Group I (Newly diagnosed acute leukemia) and group II (group I after remission induction {4 weeks}).There were also 10 patients who developed febrile attacks during the induction therapy. Fever is defined as either single temperature reading > 38.5°C or a persistent fever reading> 38°C on at least three consecutive evaluations within a 24 hour period. Twenty apparently healthy individuals served as a control group. All patients were subjected to full history taking and clinical examination. Laboratory investigations in the form of complete blood count (CBC), liver functions, kidney functions, bone marrow aspiration with immune-phenotyping at diagnosis and to assess remission, lumbar puncture and cerebrospinal fluid (CSF) examination. Assessment of the percentage (%) of peripheral lymphocytes expressing CD4+CD25+ Treg by flow cytometry technique for patients and control and for patients who developed febrile attacks. Blood culture was performed for patients who developed fever.

Methods:

Measurement of percentage of T reg (CD4+ CD25+cells).

Two milliliters of a venous blood sample were taken from each patient and control under complete aseptic conditions in an EDTA containing tube. The sample was rapidly delivered to the laboratory to assay the percentage of peripheral blood lymphocytes expressing CD4+ CD25+cells. The CD4 kit and CD25 kit were obtained from Caltag laboratories (France) for flowcytometric assessment of peripheral lymphocytes expressing CD4+CD25+ from samples of patients and controls. Gating on peripheral blood lymphocytes as a whole was done. The result of the Flow cytometer technique appears in the form of a dot plot which is divided into 4 quarters to demarcate cell populations of different phenotypes. The cells stained with FITC-conjugated monoclonal anti-human CD4 was expressed on X- axis. The cells stained with PE-conjugated monoclonal anti-human CD25 was expressed on Y- axis **(Radcliff and Jaraszeski, 1998)**.

Blood culture:

Five milliliter venous blood samples were taken from patients who developed febrile attacks under complete aseptic conditions, and inoculated in diphasic blood culture bottles using a syringe without opening the bottle. The blood culture bottles were incubated aerobically at 37°C. Then blood was sub-cultured after overnight incubation and after 48 hours, and twice weekly for two weeks. They were considered negative in case of number growth on culture media after two weeks. Blood culture bottles were obtained from Diagsera for Isolation and identification of aerobic organisms causing febrile infections among leukemic patients **(Collee et al., 1996)**.

Statistical Methodology:

Analysis of Data was performed on an IBM computer using the analytical package for Microsoft office version 11 software. Student T (paired T) and unpaired T test were used to compare quantitative variables between the studied groups. ANOVA test wad used to compare every group with other groups separately. Probability (p) value, $p > 0.05$ (insignificant),$p<0.05$ (significant),$p<0.01$ (highly significant).

Results

Laboratory findings of both patients, and control are shown in table 1 in which there was highly significant decrease in total WBC, absolute lymphocyte count in patients after induction in comparison to before induction and to control.

Table 1: Comparison between patients before induction, after induction and control as regards laboratory findings

Parameter	Before induction (n=25)	After induction (n=25)	Control (N=20)	Significance
Total white blood count x 10^3/ul	20.380± 6.783	3.888±1.204	8.285±2.424	P < 0.01
Absolute lymphocyte x10^3/ul	9.018±2.930	1.141±0.2418	3.887±1.328	P < 0.01
Hemoglobin gm/dl	7.871±1.702	9.907±1.879	11.57±1.388	P < 0.01
Platelet x10^3 / ul	23.944 ±5.985	141.15±27.645	379.80±138.17	P < 0.01

Comparison between all the studied groups as regards %of CD4+ CD25+ to total lymphocytes, and to CD4+ showed highly significant increase in % of CD4+CD25+ to total lymphocytes among patients after induction in comparison to before induction (figure 1) and highly significant increase in % of CD4+CD25+ to CD4+ among patients after induction in comparison to before induction and in comparison to control (figure 2).

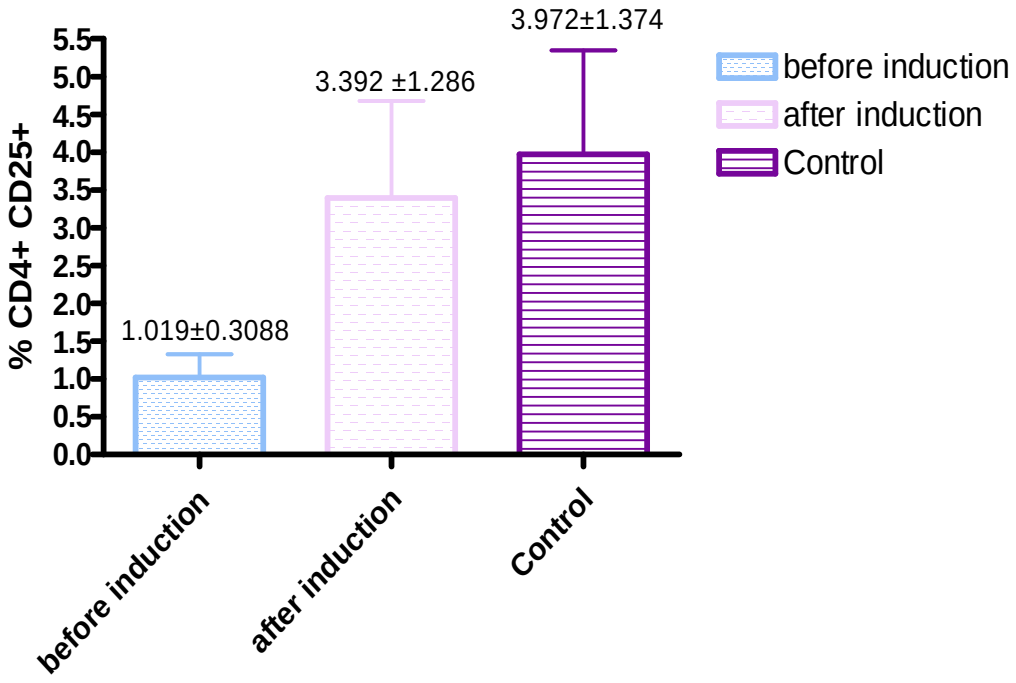

Figure 1: Patients before induction, patients after induction and control regarding % of CD4+ CD25+ to total lymphocytes

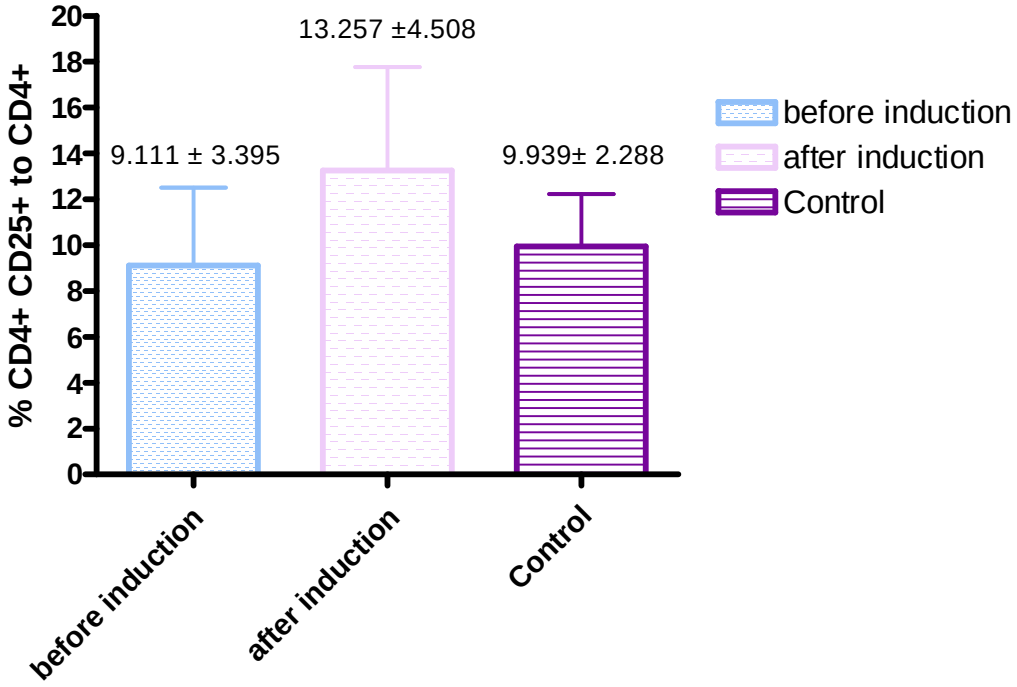

Figure 2: Percentage of CD4+ CD25+ to CD4+ between patients before induction, after induction and control

Regarding absolute numbers of CD4+CD25+, comparison between the all the studied groups are shown in figure 3in which there was highly significant decrease in absolute number of CD4+ CD25+ in leukemic patients after induction in comparison to before induction and to control.

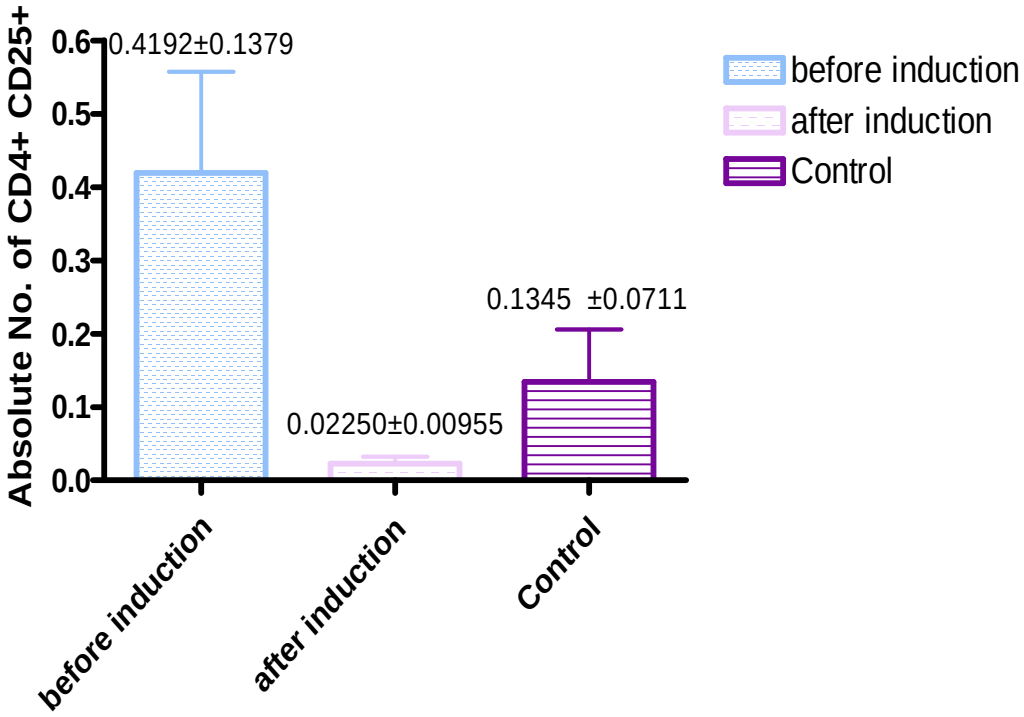

Figure 3: Patients before induction, patients after induction and control as regards absolute number of CD4+ CD25+ (x 10^{3}/ ul)

Comparison between ALL, AML and control as regards % of CD4+CD25+ (figure 4) showed highly significant decrease in % of CD4+CD25+ in ALL before induction in comparison to control, highly significant increase in % of CD4+CD25+ in AML before induction in comparison to control, highly significant increase in % of CD4+CD25 +in ALL patients after induction in comparison to before induction and significant decrease in % of CD4+CD25 +in AML patient after induction in comparison to AML patients before induction.

Table 2: Comparison between acute lymphocytic leukemia (ALL) patients before induction and after induction as regards absolute number of CD4+CD25- (x 10^3/ ul)

Group	Means ± S.D	Significance	
		T test	P value
ALL before induction	3.297 ± 1.482	8.148	P < 0.01
ALL after induction	0.4051 ± 0.09894		

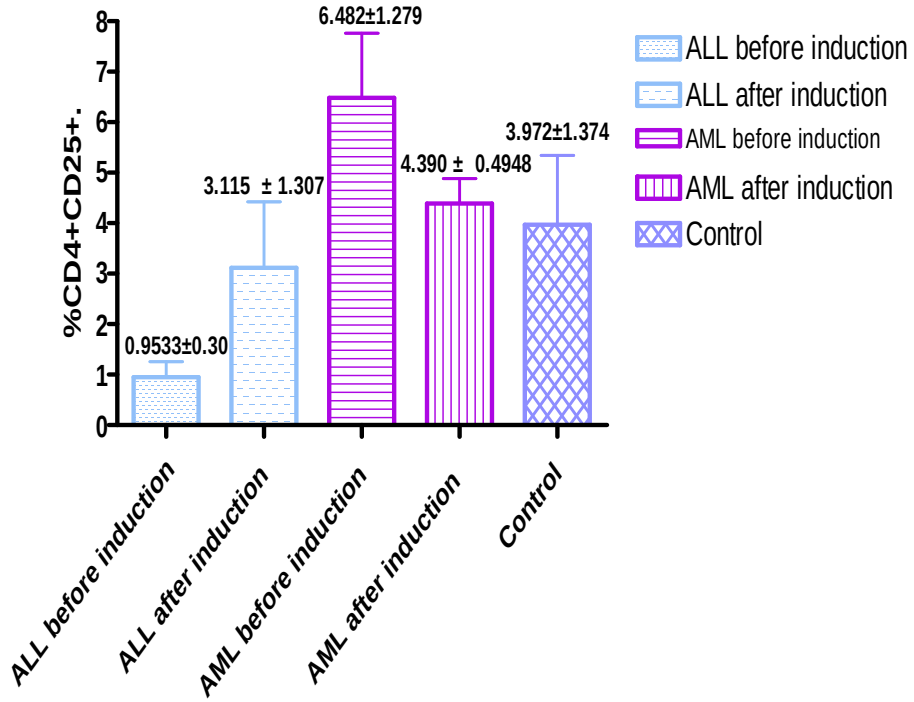

Figure 4: Comparison between acute lymphocytic (ALL) and acute myeloid (AML) patients before induction and after induction, and control as regards % of CD4+CD25+.

Regarding absolute numbers of CD4+CD25+ (figure 5), comparison between ALL, AML and control showed highly significant increase in absolute numbers of CD4+ CD25+ in ALL before induction in comparison to control, highly significant increase in absolute numbers of CD4+ CD25+ in AML patients before induction in comparison to control, highly significant decrease in absolute numbers of CD4+ CD25+ in ALL after induction in comparison to ALL patients before induction and highly significant decrease in absolute numbers of CD4+ CD25+ in AML patients after induction in comparison to AML before induction.

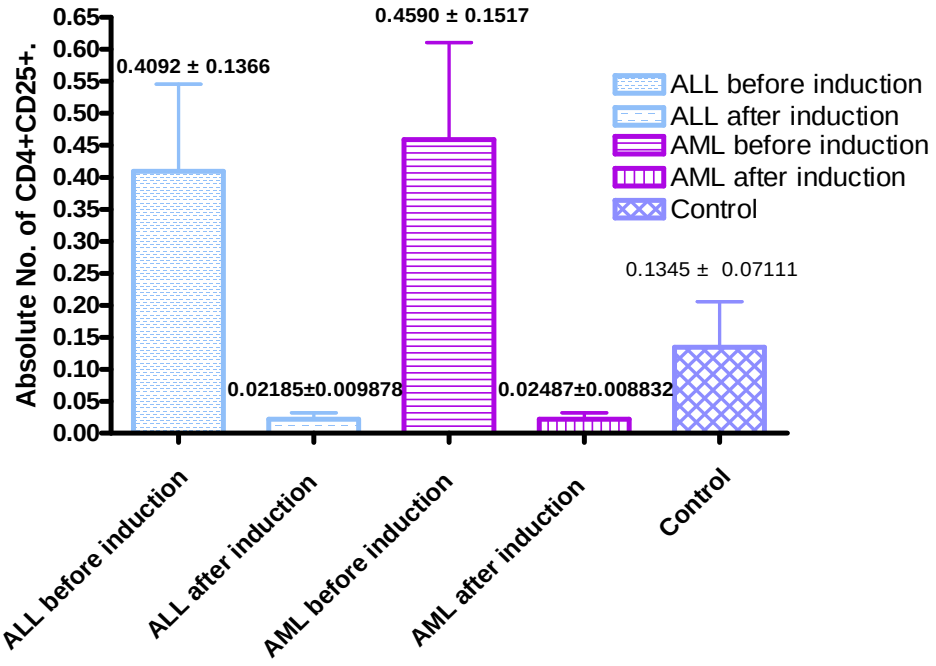

Figure 5: Comparison between both acute lymphocytic (ALL) and acute myeloid (AML) patients before induction and after induction, control as regards absolute number of CD4+CD25+.

There was non-significant increase in % of CD4+ CD25- in the cases after induction in comparison to before induction and to control as shown in figure 6, while there was highly significant decrease in absolute number of CD4+CD25- after induction in comparison to before induction and control on comparison between all the studied groups (figure 7)

There was highly significant decrease in absolute number of CD4 + CD25- in ALL patients after induction in comparison to ALL patients before induction as shown in table 2and significant decrease in absolute number of CD4 + CD25- in AML patient after induction in comparison to AML patient before induction as regards absolute number of CD4+CD25- (table 3).

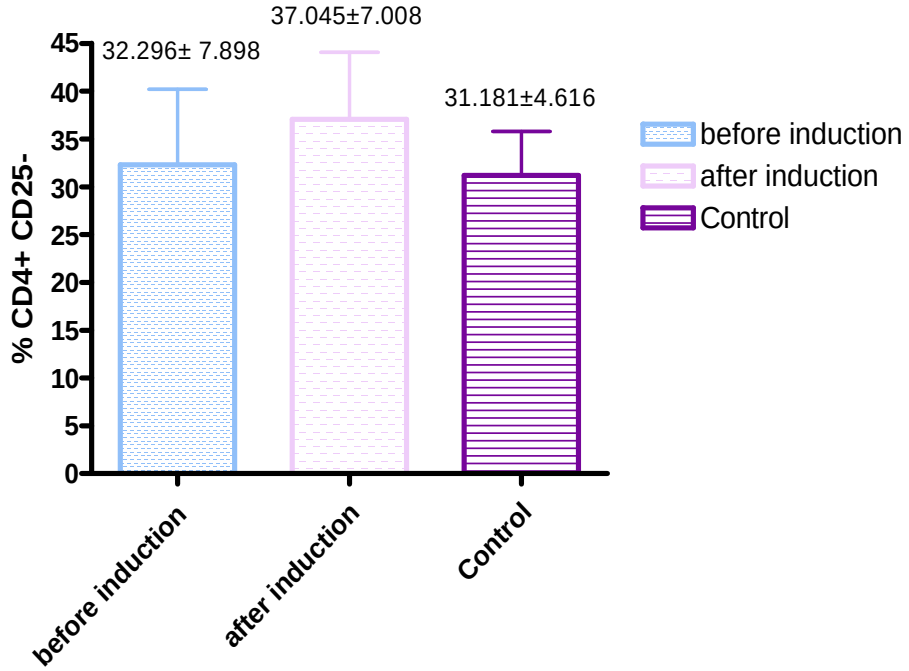

Figure 6: Percentage of CD4+ CD25- between patients after induction, before induction and controls

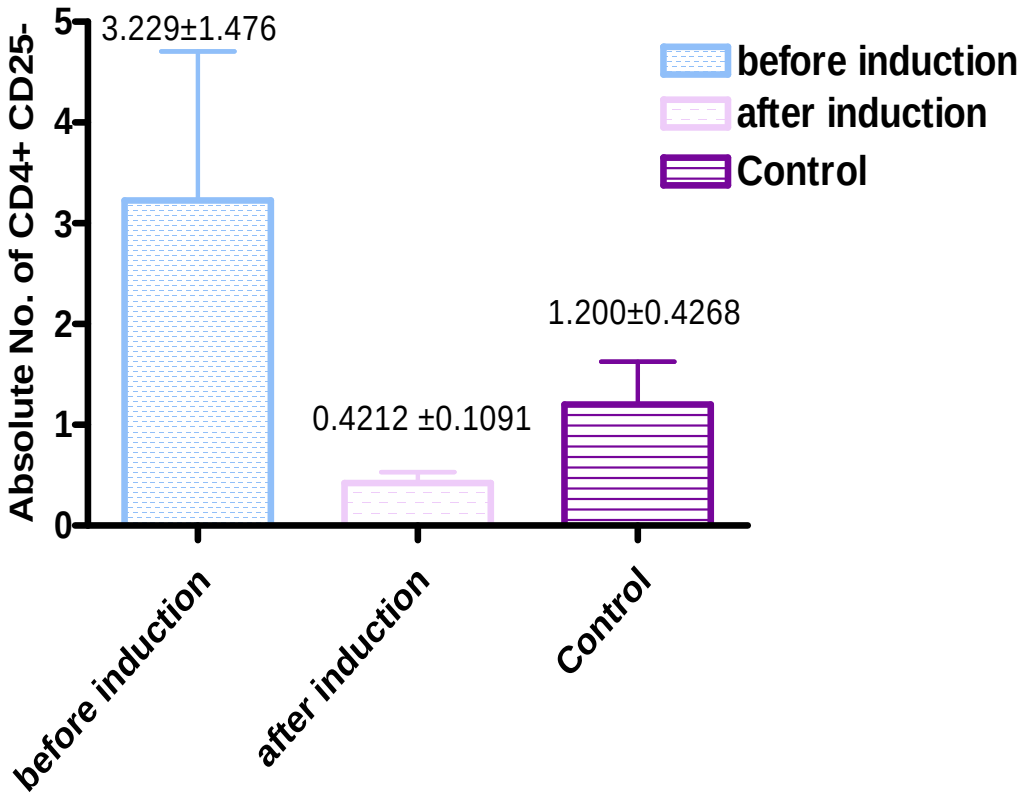

Figure 7: Patients before induction, after induction and control regarding absolute number of CD4+ CD25- (x 10^{3}/ ul).

Table 3: Comparison between acute myeloid leukemia (AML) patients before induction and after induction as regards absolute number of CD4+CD25- (x 10^{3}/ ul)

Group	Means ± S.D	Significance	
		T test	P value
AML before induction	2.956 ±1.588	3.409	P < 0.05
AML after induction	0.4792 ±0.1361		

Comparison between patients who developed fever and patients before induction as regards % of CD4+CD25+ and absolute number of CD4+CD25+ showed highly significant increase in % of CD4+CD25+ during fever in comparison to before induction (figure 8) and highly significant decrease in absolute number of CD4+CD25+ during fever in comparison to before induction (figure 9). It is worthy of note that blood culture, performed for isolation of pathogens and bacteria were negative, indicating that most infections were probably viral.

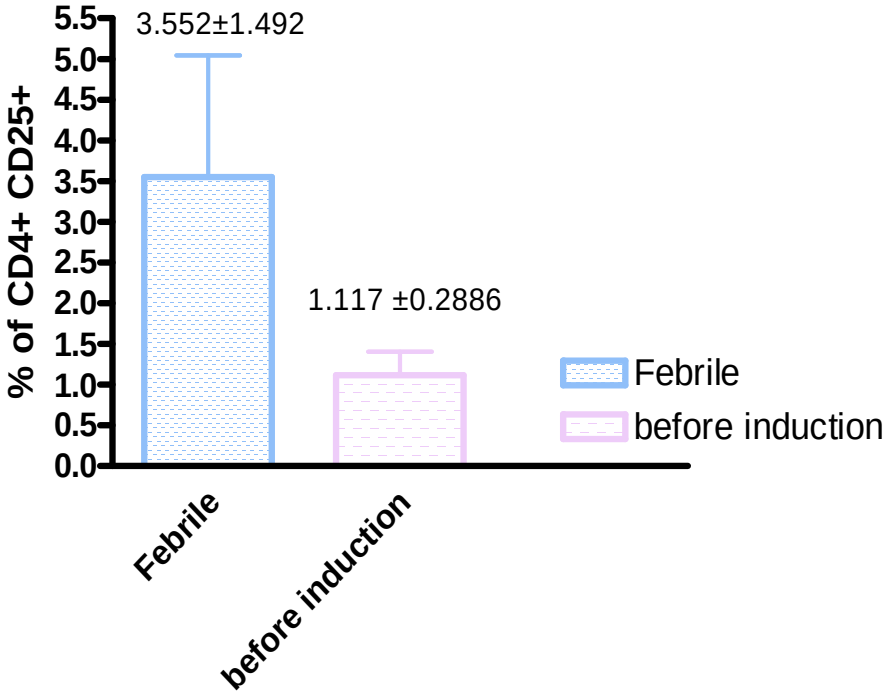

Figure 8: Percentage of CD4+CD25+ between patients who developed fever and patients before induction

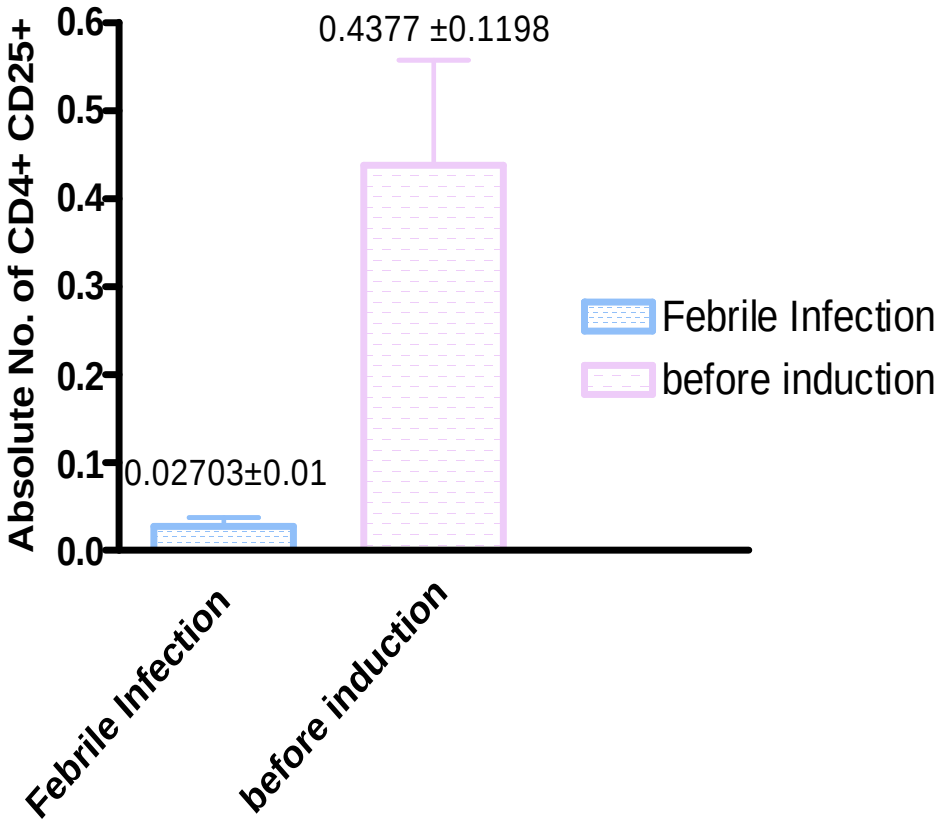

Figure 9: Patients who developed fever and patients before induction as regards absolute number of CD4+CD25+ (x 10^{3}/ ul).

Discussion

The immune system are regulated by several Tcell subpopulation (Sakaguchi, 2004). Among these cells are Treg which contribute to tumor immune-surveillance (Ha, 2009). Treg are critical in host suppression of organ autoimmune diseases. They promote a dominant state of tolerance during infections and allogenic transplantation. Treg also suppress immune responses to tumors (Turk et al., 2004). For example, several studies of murine models of human cancer indicates that CD4+CD25+ Treg cell population hinder tumor immunity (Morse et al., 2002; Strauss et al., 2007a,b). Extending these observations into human cancer, there were studies first to demonstrate that lung cancer patients had an increased frequency of CD4+CD25+Treg in their peripheral blood (Woo et al., 2001; Woo et al., 2002).Cancer patients have increased numbers of peripheral and tumor infiltrating Treg that functionally inhibit tumor-specific T cells, and predict poor survival (Curiel et al., 2004).Our ultimate goal is to open a path for mechanisms of therapeutic intervention for those patients.

This study was carried on 25 patients diagnosed as acute leukemia. Their age ranged from 11 months to 16 years, there were 18 males and 7 females. They were divided into two groups: (Group 1) 25newly diagnosed cases, 20 cases diagnosed as ALL and 5 cases diagnosed as AML and (group 2) the same 25 patients after remission induction. They were followed up until remission induction (4 weeks), and another sample was taken.10 from the 25patients developed febrile attacks during the induction therapy.

The main findings in this study was that while Treg cells, identified by expression of CD4 and CD25 (CD4+CD25+), underwent a decrease in number following chemotherapy, as was the case with other lymphocyte populations, their percentage among total lymphocyte populations and total CD4+T cells significantly increased. This finding may have implications on the role of such regulatory T cells in the pathogenesis of acute leukemia and / or the response of the immune system to this aggressive disease.

In this study, the mean absolute number of Treg in the peripheral blood was significantly higher in cases of leukemia before induction chemotherapy (0.4 19 x 10^3/ ul) compared to normal healthy controls (0.13 x 10^3/ul) (figure 3).This was manifested in cases of ALL, as well as AML (figure 5). It is possible that the higher number of Treg cells in leukemic patients occurs in response to the abnormal excessive proliferation of other lymphocyte populations in such patients. The increase in Treg cells may therefore represent the reaction of the immune system in response to excessive T cell proliferation in an attempt to empower the inhibitory response of the immune system, thus controlling disease progression. Alternatively, Treg increase in leukemic cases may reflect part of a general pattern of excessive T cell proliferation associated with leukemia. Follow up studies, to determine if large numbers of Treg recorded at the time of diagnosis is associated with better disease outcome, would favor the former possibility. Such studies are highly recommended to further elucidate these findings.

In a report by Wang et al., 2005, it was demonstrated that although the frequency of Treg was increased in AML, their apoptotic fraction was also increased. That study was conducted on 36 patients with AML. The results showed that the % of CD4+CD25+ was highly significant than in normal controls ($p< 0.001$). These results agree with our study in which we found that the % of CD4+CD25+ in AML patients was significantly higher than control, the % of CD4+CD25+ in AML was 6.482 ± 1.279, in comparison to controls which was 3.972 ± 1.374 .The cause of increased apoptotic fraction of CD4+CD25+ in AML patients is unknown. Wang et al., 2005 speculated that it could be a pathogenic procedure in development and progression of AML. It also appears that the expansion of CD4+CD25+ T cells was able to counterbalance their programmed death. This CD4+CD25+ Treg cell expansion could result from either apoptosis introduced Treg expansion or expansion of Treg repertoire to self-antigens derived from leukemia cells as reported by Mahnke et al., 2003.

After induction chemotherapy, the number of Treg decreased, as was the case with non-regulatory T cells (CD4+CD25-). CD4+T cells underwent a much more dramatic decrease in number after induction mean value before induction (3.229 x 10^3/ul), compared to after induction which was 0.4212 x 10^3/ul (figure 7). This was evident both in cases of ALL (table 2) as well as AML (table 3).These results are consistent with the findings of Mazur et al., 2005 who demonstrated that the number of lymphocytes expressing CD25 was significantly lower after chemotherapy, lower values of CD25+ may relate to most of these depleted cells being CD4+ cells, while those activated T cells are mostly CD8+ T cells. Moreover CD8+ T cells may be responsible for

suppression of CD4+T cells. Furthermore Mazur et al., 2005 demonstrated that not only abnormalities of the immune system directly associated with chemotherapy, but also there is a slow return to normal conditions during 12 months after chemotherapy.

While the total number of Treg, similar to CD4+CD25-Tcells, underwent decrease following induction chemotherapy, it is worthy to note that the decrease in the number of Treg was not that marked decrease as was the case with non-regulatory T cells (CD4+CD25-), as the mean value before induction was 0.4192 x 10^{3} / ul compared to after induction 0.0225 x 10^{3} / ul (figure 3), nonetheless the Treg increased as a percentage of total CD4+T cells after induction which was 13.257 % as compared to 9.11 % before induction (figure 2). Also Treg increased as a percentage of total lymphocytes which was 3.392 % after induction as compared to 1.019 % before induction (figure 1). This finding was also evident in cases of ALL. These results suggest that while chemotherapy is associated with an overall decrease in lymphocyte numbers, CD4+CD25+ regulatory T cells are not dramatically affected and their percentage relative to the total CD4+ lymphocyte population increases as well as to the total lymphocyte population. The fact that the percentage of CD4+CD25+T cells increased after chemotherapy is consistent with the findings of Li et al., 2007 who showed that the percentage of CD4+CD25+ T cells in patients with ALL after chemotherapy was significantly higher than that of the healthy donors and the patients with ALL without chemotherapy.

The relative persistence of Treg following induction chemotherapy may suggest that a relatively stable pool of such cells remains after chemotherapy to enhance the regulatory (inhibitory) function of the immune system on abnormally proliferating leukemia cells, in which case it would be postulated that Treg numbers positively correlate with prognosis. That is, this would suggest that the higher the number of Treg, the better the prognosis. This hypothesis, however remains to be tested in future studies.

In contradiction to our hypothesis that Treg may be associated with better disease prognosis, several mechanisms have been presented to elucidate how leukemia cells evade the immune response as discussed by Notter et al., 2001.In animal models, it had been demonstrated that there is a negative impact of Treg on both tumor specific cytotoxic T-lymphocytegeneration and tumor monitoring effectors mediated by the immune response as reported by Tanaka et al.,2002. Alternatively Fiorentino et al., 2005 had reported that disruption of Treg pathway is necessary for immunotherapeutic cure of ALL in mice. Disruption of CD25+suppressive T cells as well as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) had been shown that this combined treatment resulted in cure, through generation of immune memory cells, and TNF-α, which known to be secreted by CTL against target cells during anti-tumor immune responses.

However To our knowledge, the role of T regs in cancer immunity and the efficiency of their induction and use in cancer chemotherapy remains a conundrum. Some animal models as well as some studies in human cancers showing association between increase in Treg numbers and poor clinical prognosis strongly suggest that Tregs play a negative role in cancer progression, nonetheless, the exact mechanism remains to be elucidated (Market al., 2014). Further studies are required to determine the exact role played by T regs in cancer pathogenesis, the subset of T regs implicated and the efficacy of Tregs in cancer immunotherapy. Thorough in vivo experiments demonstrating the effects of Treg on the prognosis of leukemia will have to be performed to definitively elucidate the functional significance of Treg in the progression of leukemia and disease outcome.

Our study is supported by the findings of some groups who demonstrated similar findings in other types of cancers. Wolf et al.,2003 who performed their studies on 42 patients with different malignant tumors as lung cancer, colorectal cancer, breast cancer and gastric cancer in different stages. They found that the mean percentage of CD4+CD25+ in the peripheral blood of these patients was 2.5 fold increase than in healthy volunteers, which was highly significant. Not only the percentage, but also the absolute number were significantly increased in cancer patients.

Wolf et al., 2003 also observed the functional importance of CD4+ CD25+ expansion in cancer patients. In order to determine whether the observed increase in percentage and number of Treg in cancer patients was of functional importance, they performed proliferation assay with selected CD4+T cells from cancer patients, and from healthy controls as well. They found that CD4+T cell population was impaired in cancer patients as compared to controls. However, depletion of CD25 T cells before proliferation assay, restored the proliferative capacity of CD4+CD25- T cells. This suggests that significantly of the higher

numbers of Treg in cancer patients may be a cause, rather than a consequence of cancer pathogenesis.

Woo et al., 2002 were the first to provide evidence for increased % of Treg among tumor infiltrating lymphocytes as well as in peripheral blood. These findings go with the finding reported by Wolf et al., 2003 that there is an increase in the absolute count as well the functional importance of CD4+CD25+T cells expansion in cancer patients. These findings further support the results of Wolf et al., 2003.

Furthermore, this study in line with Cui-ping et al., 2012 who reported that the percentage of CD4+CD25+ Treg cells in the peripheral blood of B-ALL and T-ALL patients was higher compared to that of healthy individuals ($P<0.05$). Another study by Wenjuan and Yunxiao, 2013 reported that percentages of Treg cells in patients at diagnosis and during refractory/relapse were significantly higher than that in healthy controls but no significant difference in the percentages of Treg cells between patients in remission and healthy controls which disagree with our study which may be due to different ethnic group. After six cycles of chemotherapy, the percentage of Treg cells in patients who achieved complete remission was significantly lower than that in patients at diagnosis, but there was no difference in Treg frequency between refractory/relapse patients and patients at diagnosis.

The % of CD4+CD25+ Treg increased during infection in comparison to before starting chemotherapy (figure 8). That increase is due to that infection favors Treg recruitment and activates Treg. That finding agrees with McKee and Pearce, 2004 who showed that infection activates Treg through enhancement of Treg to produce certain cytokines. On the other hand the absolute number of CD4+CD25+Treg was lower than before starting chemotherapy (figure 9), that decrease may be reflective of the marked decrease in the absolute number of WBC during febrile infection in those neutropenic patients. Blood culture, performed for isolation of pathogens and bacteria were negative, in this study which agrees with Uys et al., (2000) who showed that viral isolates accounts for about 38% in patients with febrile neutropenia. Bacterial isolates accounted for 22% of these episodes. The most frequent isolate was influenza virus as shown by Cotton et al., 1984. Unlike Uys et al., 2000 who showed that Herpes simplex virus was the most common isolate.From these previous studies it had been evident that viruses are found in a significant number of patients with febrile attacks. Viruses could be identified more often than bacteria, and may account for a significant percentage in febrile neutropenia in childhood malignancies.

The current study shows that numbers of Treg is increased in leukemic patients than healthy individuals. These findings provide implications for therapeutic intervention. If that increase correlates with prognosis, the higher the number the better the prognosis, therefore there must be therapeutic strategies to enhance Treg populations by using Treg as a vector in tumor immunotherapy. It could be achieved through infusion of exogenous Treg population expanded in vitro. IL-2 could also be used as it was described to be the main Tcell growth factor. However, if the increase in Treg number has a negative impact on the effectiveness of immunotherapies of cancer. Therefore, depletion of Treg may become a successful anticancer strategy. In conclusion, manipulation of Treg should be added to the therapeutic interventions for enhancing tumor immunity in humans. Further experimental studies in vivo showing the effects of Treg on prognosis of leukemia are strongly recommended. Implications for Treg to be used as a therapeutic intervention in tumors due to its pivotal effects in tumor immunity may be important as a mechanism of tumor therapy. Viruses should be considered as a major cause in febrile neutropenia in patients with malignancies, as it accounts for a significant percentage in their febrile attacks.

Conflict of interest:

The authors declare that no funding or grant was received for the study, and that they have no conflict of interest, financial or personal relationship related to the study.

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