Efficacy and Tolerance of Fludarabine and Cyclophosphamide (FC) Combination Regimen in advance stage of Chronic Lymphocytic Leukemia

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Abstract

Background: based on data suggesting synergistic activity of fludarabine (F) and cyclophosphamide (C) combination therapy. This study was conducted to assess the efficacy and tolerability of this combination chemotherapy in Iraqi adult patients with advanced chronic lymphocytic leukemia (CLL), reflected by better response rate, treatment free survival and overall survival benefit.

Patient and Methods: A prospective study carried out in Baghdad teaching hospital and national hematology center in Baghdad between February 2005 and fabruary 2009. It included 64 Iraqi patients aged between 39-77 years old with advanced stage CLL. Written informed consent wasobtained from all patients prior to start of therapy. They received FC combination therapy (fludarabine 25 mg/ m2 plus cyclophosphamide 250mg/ m2 for 3 days intravenously, repeated every 28 days). Treatment was administered for 6 courses.

Results: Out of 64 CLL patients with Binet stage B&C who were included in this study, 41(64.1%) patients were male and 23 (35.9%) patients were females (M:F ratio1.7:1) with median time of follow-up 26 months. median age was 59.9 years. Forty- eight (75%) patients were previously untreated, of them 22 (45.8%) patients were stage B and 26 (45.1%) patients were stage C while the other 16 (25%) patients with CLL were previously treated with an alkylating agents, of them 7(43.7%) of stage B and 9 (56.2%) with stage C. This combination chemotherapy resulted in 39.1% complete remission and 39.1% partial remission. The two years median treatment-free survival was 90% with the median duration of response was 18 months. there was a significant difference ($p<0.005$) between the different Binet stage group (C&B) and degree of response, with better response rate in those with stage C than those with stage B. the overall response rate was 88.5%, 65.5% for stage C and B respectively.The major toxicity (grade 3-4) were nausea and vomiting while the myelosupression of grade 1&2 for leucopenia and neutropenia occur in 19%, 14% respectively.

Conclusion: Fludarabine and cyclophosphamide combination regimen is an effective therapy for patients with advance CLL with high response and complete remission rate in those untreated and previously treated CLL patients, with good tolerability to this combination.

Keywords: Efficacy, Fludarabine, Cyclophosphamide, CLL.

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Introduction

Chronic lymphocytic leukemia (CLL) is the classical leukemia of the elderly and the treatment often must be tailored to the patient's fitness level and ability to tolerate more toxic combination therapies. As a consequence, the therapy of CLL becomes increasingly personalized, requiring a detailed knowledge about the different diagnostic and therapeutic options[(1)] .

Three purine analogues are currently used in CLL:fludarabine, pentostatin, and cladribine. Fludarabine remains by far the best studied compound of the three in CLL, produces superior overall response (OR) rates compared with other treatment regimens containing alkylating agents or corticosteroids[(2)].

Overall response rates of 75–80% and 50%have been reported in treatment-naive and previously-treated patients, respectively. Complete response (CR) rates with fludarabine monotherapy are typically 15–20% in previously treated patients and 40–50% in treatment-naive patients.

Three randomized trials have shown that the addition of cyclophosphamide to fludarabine clearly improves the CR and OR rate and progression free survival(PFS) as compared with fludarabine monotherapy. An additional important result of these trials was that FC did not increase the rate of severe infections despite inducing more grade 3 and 4 neutropenias[(3)].

Fludarabine inhibits the repair of DNA damage caused by agents such as Mitoxantrone and cyclophosphamide. A synergistic effect has been demonstrated between fludarabine and cyclophosphamide[(4)].

The major toxicities are hematologic and immunologic. Neutropenia is noted in approximately two-thirds of treated patients with advanced disease, although this usually is not dosed limiting. The major morbidity associated with fludarabine is immune suppression. Fludarabine produces a pronounced decrease in the number of blood T cells, especially CD4+ T cells, that often persists for more than a year after therapy[(5)].

Treated patients apparently have an increased incidence of infection with opportunistic organisms, including herpes simplex, herpes zoster, *L.* monocytogenes, and Pneumocystis jiroveci (formerly called Pneumocystis carinii). Patients also may experience reversible neurologic toxicity, even after receiving the standard dose of fludarabine[(6)].

Patients treated with fludarabine have been noted to have an increased incidence of new onset autoimmune diseases, such as autoimmune hemolytic anemia, immune thrombocytopenia, and pure red cell aplasia[(7)].

Finally, CLL patients treated with fludarabine also may develop transfusion-associated graft-versus-host disease, possibly reflecting the overall impairment to the host immune system that is induced by this drug[(8)].

Aims of Study

1. To assess the efficacy and tolerability of combination therapy with fludarabine and cyclophosphamide in patients with advance stage of chronic lymphocytic leukemia

(CLL) in previously treated and untreated patients.
2. To assess the treatment free and overall survival in CLL patient who are receiving FC regimen therapy.

Patients and Methods

This single arm prospective study was carried out in Baghdad teaching hospital and the National center of hematology in Baghdad between February 2005 to February 2009.During this period, 64 patients of chronic lymphocytic leukemia with stage B and C according to Binet classification were included, Patients with Binet stage Awere excluded. The study was approved by the Institutional Ethics Committee. A written informed consent was obtained from all patients prior to start of therapy. History and physical examination, complete blood counts, differential and platelet counts, liver and renal function studies, bone marrow aspiration and biopsy were done for all patients. The diagnosis of CLL was done according to the criteria and recommendation of the international workshop of CLL (IW-CLL) with sustained peripheral blood lymphocyte count of $>10x10^9$ \L, most of the cells being mature appearing lymphocyte and bone marrow aspirates showing greater than 30% lymphocyte with normal renal and liver functions. Flowcytometer was not available to be used in the diagnosis of CLL.

Treatment protocol which given to patients included fludarabine dosed at 25 mg/m^2, administered intravenously daily over 30 minutes for 3 days, plus cyclophosphamide 250 mg/m^2 administered intravenously daily over 30 minutes for 3days, regimen were repeated every 28 days for maximum of 6 courses. Delaying of therapy for 1-2weeks was permitted if absolute neutrophil count pre course therapy was less than $1500/mm^3$.

Response was assessed by peripheral blood count, bone marrow aspirate and trephine biopsy at the end of the six courses of FC regimen. The criteria of response was according to national cancer institute working group (NCIWG) proposal, three types of response were defined: complete response including nodular partial remission, partial response and no response.

Complete remission was defined as normal findings on physical examination, disappearance of all symptoms and normal blood count which was defined as lymphocyte countlower than 4×10^9/L, neutrophil count higher than 1.5×10^9/L, platelet count higher than 100×10^9/L, hemoglobin level higher than 11 g/dL, and bone marrow lymphocyte percent ageless than 30% on aspiration and biopsy. Moreover, imaging diagnostics by chest X-ray and abdominal ultrasound,which had to show negative findings for lymph node enlargement (enlargement was defined as greater than 1cm), splenomegaly and hepatomegaly. Partial remission wasdefined as 50% reduction of all measurable disease manifestationsin physical and imaging examination and more than 50% improvement of all abnormal blood counts. No response was defined as those patients showing no regression neither in lymph nodes, spleen nor in liver size or improvement in blood counts.
Trimethoprim was given twice weekly as prophylactic therapy from starting point of protocol till 6 months after end of last course of therapy and absolute lymphocyte count $\geq 1000/ mm^3$.All data on toxicities were available for only 47 patients of 64 ,bothmild (grades 1 and 2) and severe (grades3 and 4)

side effects were recorded according to common toxicity criteria (CTC 1.0).

Statistical Analysis

Associations between patient characteristics and response outcome were evaluated by chi square test. Distributions of survival and time to progression were estimated by the method of Kaplan and Meier. Overall survival were measured from first day of chemotherapy until death; deaths from all causes were included, while progression free survival was calculated from first day of chemotherapy to the time of disease progression and failure of treatment or death. Response rates were calculated for all patients after they complete their cycles of therapy till time of relapse or death. All data analyzed statistically by using SPSS13.0 (Chicago.IL).

Results

Out of 64 patients who were included in this study, 41 (64.1%) patients were male and 23 (35.9%) patients were females (M: F ratio1.7:1) with median time of follow up 26 months. Age ranged from 39 to 77 years with median age 59.9 years. Detailed characteristic are shown in table 1.

Of 64 patients with CLL, 48 (75%) patients were previously untreated, while 16 (25%) patients of CLL were previously treated with alkylating agents.
Response rate to FC regimen according to NCIWG criteria are shown below in table 2, with response rate (complete and partial response) was 78.2% and median duration of response is 18 monthes±11.21.
The response rate(for CR and PR) between two different groups of CLL patients shows higher response rate in untreated than in previously treated patients, 83.4%, 62.5% respectively, but this was statistically not significant (P value 0.176) as in table 3; while the difference in the response rate through different stages of CLL patients was show higher response rate (88.5%) among group C patients than group B CLL patients (65.5%), despite the CR rate was prominently was higher for stage B than C, with significant P value was 0.02 as shown in table 4.

Table 5 shows that 25of 26 (96.1%) patients with stage C, they are naïve and respond to therapy, while 6 of 9 (66.6%) patients with stage C previously treated had responded to therapy which was statistically significant p value 0.032.

Only 4 (6.2%) patients died during the study, three of them were died during therapy courses due to severe infections while the other one died because of cerebral hemorrhage because of severe thrombocytopenia. The 2 years overall survival was about 95% with median duration of follow up 26 months. The median progression free survival for those patients was not reached and 2 years progression free survival was 90%. Data available for FC regimen induced toxicity was recorded in only in 47/64 CLL patients and as shown in table (6), this evaluated according common toxicity criteria.

Table 1.patients characteristics at time of entry

Characteristics	
No. patients	64
Median age, yr (range)	59.9 (39 – 77)
Male, no. (%)	41(64.1%)
Female, no. (%)	23(35.9%)
M:F ratio	1.7:1
Binet stage, no. (%)	
B	29(45.6%)
C	35(54.4%)
Disease status no. (%)	
Untreated (naïve)	48(75%)
previously treated	16(25%)
Leukocyte count x 10^9/L (range)	8.33 (1.2 -44.1)
Hemoglobin level, g/dL (range)	10.4 (7.7-14.3)
platelets count, x10^9/L (range)	165 (78-353)

Table 2. Degree of remission in different responder patients

Response state	**NO.**	**%**
Complete response(CR)	25	39.1%
Partial response(PR)	25	39.1%
No response(NR)	14	21.9%
Total	64	100%

Table 3. Degree of the response between those previously untreated and treated patients CLL patients

Type of patients	**Degree of response rate**			**Total**
	CR	**PR**	**NR**	
Previously untreated	21(43.8%)	19(39.6%)	8(16.7%)	48(100%)
Previously treated	4(25%)	6(37.5%)	6(37.5%)	16(100%)

Table 4. Patients differences between response rate and different CLL stages

stage of CLL	**State of response**			**Total**
	CR	PR	NR	
B	14(48.3%)	5(17.2%)	10(34.5%)	29(100%)
C	11(31.4%)	20(57.1%)	4(11.4%)	35(100%)

Table 5. Difference in response between two stages

stage	**Naïve patients**		**Previously treated**		**Total No.**
	Respond	**Not respond**	**Respond**	**Not respond**	
Stage B	15/22(68.1%)	7/22(38.1%)	4/7(57.1%)	3/7(42.1%)	29
Stage C	25/26(96.1%)	1/26(3.8%)	6/9(66.6%)	3/9(33.3%)	35

Table 6. undesirable side effect of FC regimen in CLL patients

UNDESIRABLE EFFECT	Grade 1-2	%	Grade 3-4	%
General condition	30	63.8	14	29.7
Anemia	38	80.8	9	6.3
Leucopenia	21	44.6	6	12.7
Neutropenia	23	48.9	0	–
Thrombocytopenia	31		0	–
Infection	39	82.9	0	–
Fever	28	59.5	9	6.3
Infection	7	14.8	2	4.2
Vomitting	1	2.1	0	–
Stomatitis	15	31.9	0	–
Diarrhea	6	12.7	0	–
Bilirubin	0	0	0	–
SGOT/SGPT	0	0	2	4.2
Peripheral neuropathy	9	6.3	0	–

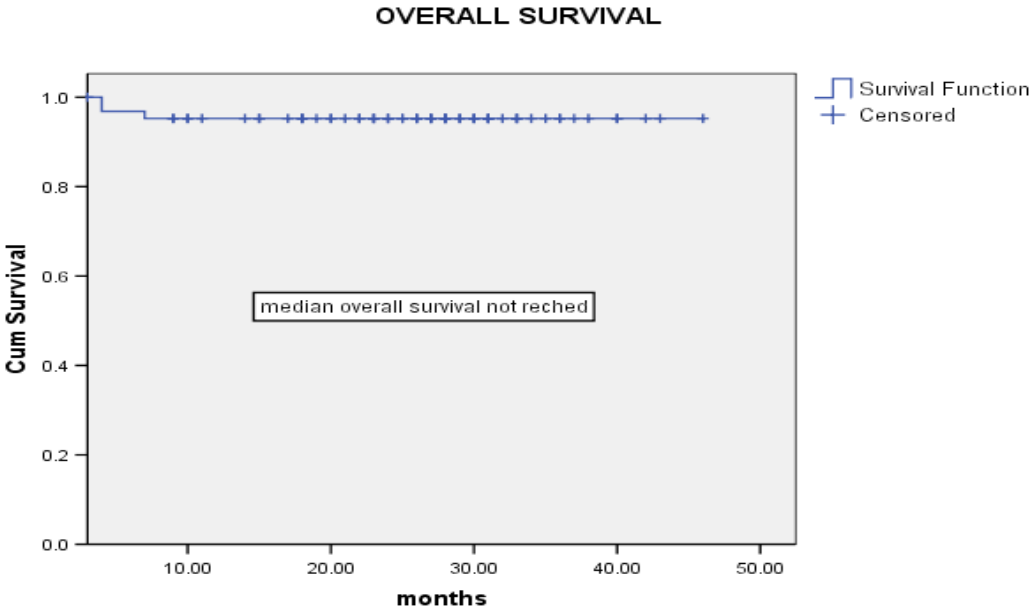

Figure 1. Overall survival using the Kaplan-Meier method

Figure 2. Progression free survival of the randomized patients

Discussion

The nucleoside analogue, fludarabine, is very effective drugs in the treatment of CLL, This efficacy has been demonstrated in several studies with higher remission rate than conventional chemotherapy in advanced CLL, particularly in patient resistant to alkylating agent[(9)].

The FC regimen response showed overall response rate 78.2% of these 39%, 39% and 21.9% had complete response, partial response and no response respectively, without significant difference in response rate among those previously treated and untreated patients 62.9% versus 83.4%**(**P-value 0.176). This is comparable to the response rate of multicenter phase III study initiated by ECOG and later amended to include Cancer and Leukemia Group B (CALGB) and Southwest Oncology Group with response rate to combination therapy74.3% and complete response23.4%[(10)].

German CLL phase II study group evaluated the efficacy and toxicity of a combination of fludarabine and cyclophosphamide (FC) in patients with B-cell CLL in 36 patients with overall response rate (RR) 90.6%, with 13.9%complete remission (CR) and 66.6% partial remission (PR). This high response rate of the FC regimen was independent of the treatment status prior to the study. The separate analysis of response for untreated and pretreated patients revealed no significant difference, with a response rate of 85.7 and 94.4% respectively [(9)]. The German CLL group phase III study by Eichhorstetalalso yields higher response rate 94% and similar CR 24% to U.S intergroup[(11)].

In this study there was significant difference ($p < 0.02$) in response rate between stage B&C where 88.5% of patients with stage C had response (partial and complete response) while 65.5% of stage B were experience this response, this is comparable to **German CLL Study Group** results which showed benefit of CLL patients with stage C disease more from

stage B patients who used FC regimen than from fludarabine monotherapy[11]. There was no significant difference in the response rate between previously treated and naive patients.

O'Brien etal showed that fludarabine and cyclophosphamide produced ≥ 80% response rates in all patients not refractory to fludarabine at the start of therapy as well as a 38% response rate in patients who were refractory to fludarabine. The complete remission (CR) rate was 35% in previously untreated patients, which was not significantly different from the CR rate in historical control patients treated with single-agent fludarabine[6].

Only four (6.25%) patients enrolled in this study died before complete their courses therapy, all deaths were of stage C and previously heavily treated CLL patients; the cause of death was severe infection in three patients and in tracerebral hemorrhage in the 4th one.

The median observation time of 26 months was too short to validate a survival difference between pretreated and untreatedpatients. Two-year overall survival is currently estimated as 95% for patients randomly assigned to FC.

Toxicity data were documented in only 47patients, 85% of patient experience grade 3-4 toxicity documented as nausea and vomiting, this may be due to lack of treatment with antiemetic especially ondasteron. Anemia and neutropenia was mostly of grade 1 and 2 toxicity in 80.8%, 48.9% respectively. Grade 3-4 Leucopenia and neutropenia were demonstrated only in 19% and 14% respectively, and this reflected in that no serious infections were documented and this raised the point that may be due to routinely use of prophylactic treatment against opportunistic infection with antiviral and antifungal, which might reduce incidence of serious infection, However grade1-2 infections were registered in 82%. Hallek et al showed that no severe infection in CTC grade 3 and 4 was observed, despite the frequent occurrence of CTC grade 3 and 4 neutropenia in more than two-thirds of patients (69.4%). Nevertheless, the results indicate that using a lower cyclophosphamide dose in the FC regimen might be advantageous with regard to the incidence of severe infections,(9) and this is similarly was observed by Eich horst et al, in which he showed that FC regimen caused thrombocytopenia in 15.6% and leukocytopenia 55.5%, but did not increase the number of severe infection[11].

In conclusion fludarabine and cyclophosphamide combination regimen is an effective therapy for patients with advance CLL with high response and complete remission rate in those untreated and previously treated CLL patients, with good tolerability to this combination.

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فعالية وتحمل الجمع بين علاح الفلودارابين مع السيكلوفوسفاميد في علاج المراحل المتقدمة من ابيضاض الدم اللمفاوي المزمن

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الملخص

الخلفية: استنادا إلى البيانات السابقة بخصوص التازر الفعال بين علاح الفلودارابين مع السيكلوفوسفاميد في علاج المراحل المتقدمة من ابيضاض الدم اللمفاوي، تم عمل هذه الدراسة، والهدف منها تقييم فعالية وتحمل هذا العلاج الكيماوي في المرضى البالغين المصابين بابيضاض الدم اللمفاوي المزمن في المركز الوطني لأمراض الدم ومستشفى بغداد التعليمي في بغداد العراق، من خلال معرفة نسبة الاستجابة الشاملة ونسبة بقاء المرض دون علاج.

طرق الدراسة: دراسة مستقبلية أجريت في مستشفى بغداد التعليمي والمركز الوطني لأمراض الدم في بغداد بين شباط 2005 وشباط 2009. وشملت 64 مريضا من الذين تتراوح أعمارهم بين 39 و77 سنة مع مرحلة متقدمة من ابيضاض الدم اللمفاوي المزمن. تم الحصول على الموافقة المسبقة الخطية من جميع المرضى الذين شملتهم الدراسة قبل البدء بالعلاج. تم إعطاء علاح الفلودارابين بجرعة 25 ملغم للمساحة السطحية مع السيكلوفوسفاميد 250 ملغم للمساحة السطحية لمدة 3 ايام عن طريق الوريد، ويكرر هذا العلاج كل 28 يوما ولمدة 6 دورات.

النتائج: من أصل 64 مرضى الذين كانوا في مرحلة ب وج حسب تقسيم بينت، كان هناك 41 بنسبة (64.1%) من الذكور و23 بنسبة (35.9%) من الاناث، وكان متوسط الوقت اللازم للمتابعة 26 شهرا. وكان متوسط العمر59.9 سنة. وكان ثمانية وأربعون (75%) من المرضى من الذين لم يأخذوا اي علاج من قبل، منهم 22 (45.8%) من المرضى كانوا في مرحلة ب B المرحلة و26 (45.1%) من المرضى كانوا في مرحلة ج، في حين 16 (25%) من المرضى كانوا قد عولجوا بالعقاقير الكيمياوية سابقا، منهم 7 (43.7%) من المرضى كانوا في مرحلة ب، و9 (56.2%) من المرضى كانوا في مرحلة ج. أدى استعمال هذا الجمع بين العلاجين إلى شفاء تام بنسبة39.1% وشفاء جزئي بنسبة 39.1%. وكانت نسبة الاستجابة الشاملة والبقاء على قيد الحياة لمدة سنتين 90% وكان متوسط المدة الزمنية للاستجابة 18 شهرا. كانت هناك علاقة مهمة بين مرحلة بينت ب وج ودرجة الاستجابة. مع معدل استجابة أفضل في مرحلة ج مقارنة بمرحلة ب. ومعدل الاستجابة الكلي كان 88.5 و65.5 لمرحلة ب وح على التوالي. سمية العلاج الغالبة درجة (3 و4) شملت الغثيان والتقيؤ، بينما شملت درجة 1 و2 لنقص الكريات البيض وقلة العدلات، حيث وجد في 19% و14 % على التوالي.

الخلاصة: الجمع بين علاح الفلودارابين مع السيكلوفوسفاميد هو العلاج الفعال لمرضى المراحل المتقدمة من ابيضاض الدم اللمفاوي المزمن مع نسبة استجابة عالية وشفاء تام في المرضى الذين لم ياخذوا اي علاج من قبل والذين سبق ان عولجوا بالعقاقير الكيمياوية مع تحمل جيد لهذا الجمع.

الكلمات الدالة: فعالية، الفلودارابين مع السيكلوفوسفاميد، ابيضاض الدم اللمفاوي المزمن، العلاج الكيماوي.