PRELIMINARY ASSESSMENT OF EFFICACY OF *NIGELLA SATIVA* SEEDS IN ACUTE LYMPHOBLASTIC LEUKEMIA IN LOCAL CHILDREN

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Summary

Newly diagnosed acute lymphoblastic leukemia (ALL) patients were randomly divided into Groups I-III; having 21, 20 and 21 patients each, respectively. Ten age, sex and socio-economically matched healthy subjects were included for reference. Blood samples were taken for hematological parameters. In all groups these procedures were repeated after every 0.5 month till 6 months (including first 3 months of completion of induction chemotherapy followed by maintenance therapy. Due to various reasons, 48 subjects (16 in each) completed the study and their data were analyzed. In conventional therapy (Group I), daunorubicin (1.5mg/kg,i/v weekly), vincristine (.4mg/kg i/v twice weekly) and prednisolone (5 mg/kg per day orally in 3 divided doses) were prescribed to patients of ALL as induction therapy for a period of 3 months. In Group II, L-asparaginase was administered in addition to Group I therapy in 100u/kg, i/m twice weekly dose. Powdered *Nigella sativa* seeds were given to Group III patients in addition to conventional therapy (without L-asparaginase) in 40 mg/kg orally in two equally divided doses.

The data showed that prognosis was good in all groups and was similar in male and female. Remission rate (RR) was affected by increasing age and survival was better in younger patients. The prognosis was worse in patients with high (>50000/mm^3) initial WBC counts. RR was negatively affected by severe anemia (Hb<8.0g/dl) which showed positive correlation with Hb level. ESR was markedly raised in majority of patients at presentation but remained high even after induction therapy. The RR and survival time were better in patients having blood ESR between 10 to 100 mm/1st hr.

Survival rate was also affected by blast cell count but platelet count showed no correlation with prognosis. Group1 treatment and L_2 & L_3 morphologic subtypes carried worse prognosis and better outcome was seen in Group III with L_1 morphology. When compared, complete remission was obtained by all treatments, better by II and III**.** Four patients died and did not show remission were 2 males and 2 females and 3 were above 12 and one child was below 12 years. In Group II, total cost per subject was highest (Rs. 75,000 costlier than Group I while Group III treatment was only Rs 240 costlier than Group I. Group II treatment with L-asparaginase as additional agent has resulted in prolongation of BT and CT in ALL patients. The rise in glucose in Group II was more than others while ALT, ALP and amylase were higher in Group II than Groups I and III. Group III treatment with conventional drugs + N*igella sativa* seeds proved best among the three treatments. It was also observed that determination of age, sex, cell morphology, DLC, Hb, ESR and blood glucose, urea, bilirubin, ALT, ALP, amylase levels were also helpful in predicting toxic/side effects of cytotoxic drugs. Acute pancreatitis, hyperglycemia, and increase in ALT, ALP, amylase levels and prolongation of BT and CT were more prevalent in 2nd group receiving L-asparaginase while such changes were not observed in other groups. It is conceivable, therefore, that *Nigella sativa* seeds could help in treating acute lymphoblastic leukemia in children when given in combination with other cytotoxic drugs

Keywords: Acute lymphoblastic leukemia, ALL, *Nigella sativa* seeds, L-asparaginase, induction treatment, toxic effects

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Introduction

Cancer is 2nd most common cause of mortality in USA after cardiovascular diseases (1). All ages develop cancer and a wide variety of organs are affected (2). The incidence increases with age. Apart from individual sufferings, the economic burden to the society is immense (1). Each year about one million individuals hear the news that they are suffering from cancer and there were 538,000 deaths (about 23% of total deaths) in 1994 from cancer in USA (3). Its incidence has increased rapidly in Pakistan too and about 20,000 people in Pakistan suffer from this disease each year (4). In Pakistan, most frequent cancers in adults are that of breast, ovarian, lung, and oral whereas in children most frequent cancers are found in lungs, large bowel, breast and blood in the descending order (3).

Leukemia is an uncontrolled tumorous growth of abnormal nonfunctional white blood cells in bone marrow and their outpouring into blood (5). It is a malignant neoplasm of hemopeotic stem cells characterized by diffuse replacement of bone marrow along with blood. The cancerous growth involving stem cells of WBCs origin results in development of leukemias like myelomonocytic leukemia or myeloid leukemia and lymphocytic leukemia which may have a short, acute, rapidly progressive course or a slow chronic, insidious onset. In acute myeloid leukemia, prevalence of blast cells in peripheral blood may be equal to 30% or more of total WBCs (6). When lymphocytes are involved, the condition may have acute onset usually in early age with severe generalized symptoms and blood and bone marrow show diffuse infiltration of lymphoblasts. This condition is called acute lymphocytic or lymphoblastic leukemia (ALL) while in rest 15% of cases, the course is long with usually late age onset in the absence of blast cells in blood, this is called chronic lymphocytic leukemia (3).
Leukemia has a latent period of 5-7 years whereas solid tumors have more than 40 years (7). Diagnosis of leukemia is easy from the history of patient, physical examination and laboratory findings from bone marrow and peripheral blood films. Once the diagnosis is established, Oncologist start best known choice drugs for treatment (3). An ideal anticancer would eradicate cancer cells without harming normal cells. However, currently so such agent is available that meets this criterion. So trials should involve weighing benefits against toxicity in a search for a favorable therapeutic agent. It has been suggested that treatment of ALL should consist of 2 phases, first phase is induction of remission (greater than a 3 log cell kill), the therapy will start from day one to usually 40 to 90 days which requires consolidation in high risk patients like male adults, more resistant to therapy.

Conventional drugs used for start of induction treatment include daunorubicin, vinca alkaloids and steroids. L-asparaginase (LA) has also been used for synergistic activity. The 2nd phase of treatment include maintenance therapy with 6-mercaptopurine, methotrexate and cyclophosphamide for a period of 18 months to 3 years (8,9). Moreover, *Nigella saliva* seeds already used in traditional medicine since centuries (10) were included in the present trial in combination with conventional therapy of ALL patients. Thus we determined efficacy of conventional treatment and try to replace, if possible, L-asparaginase with *Nigella saliva* seeds in the induction treatment of acute lymphoblastic leukemia in children.

Materials and Methods

Sixty two subjects of already diagnosed patients of acute lymphoblastic leukemia (ALL) of ages between 2-18 years were selected randomly from those visiting Department of Oncology, Allied Hospital, Punjab Medical College, Faisalabad. Ten normal subjects were also selected from local population. They were having age, sex and socioeconomic status matched with ALL patients. Selection criteria were total TLC, DLC and cell morphology of peripheral blood films and the confirmed by bone marrow examination (3, 11). At the time of diagnosis, out of 62 ALL patients 20 (32.2%) had features of L_1 type and L_2 type were in 33(53.2%) patients. In remaining 9 (14.5%) patients were of L_3 subtype.

The selected subjects were randomly divided into 3 groups: Group I received conventional treatment and included 21 cases. Group II received conventional treatment along with LA and were 20 cases. Group 111 patients received conventional treatment with powdered *Nigella saliva* seeds and were 21 cases.
The blood samples from all patients and controls were collected at zero time and after every 15 days till 6 months. TLC, DLC, white cell morphology, blast cells, clotting time and platelets counts were made (12), bleeding time (13), hemoglobin and ESR (14), fasting blood glucose (15)), blood urea (16), blood bilirubin (total, direct & indirect) by methods of others (17). Blood ALT were assessed (12), along with blood alkaline phosphatase and blood amylase too(16). The duration of treatment for induction of remission was three months. In order to see prognosis of disease and development of side effects during and after treatment, hematological/biochemical tests were performed till 6 months. The induction of remission treatment was completed as per recommendations (18). After each 15 days, all tests mentioned above were again performed.

Repeated samplings could be completed only in 48 patients. Due to various reasons, remaining 14 subjects could not complete study as they failed to participate in follow-up and 4 died during remission phase. Thus complete data could be obtained from 48 patients and analyzed to evaluate efficacy of the 3 test treatments. MStatc computer program was used to analyze data by one way analysis of variance technique. The completely randomized design was used and Duncan's Multiple Range applied to differentiate the treatments effects between groups (19).

Results and Discussion

Out of 62 ALL patients 10 could not come for follow up. Then out of 52 remaining cases, 4 died and 48 completed induction therapy course. Thus, remission rate was 48/52 (89%) in total and 16/18 (87%) in Group I, 16/17 (92%) in Group II and Group III. Similar results have been reported with the addition of L-asparaginase given additionally (18). In addition, *Nigella saliva* seed was given additionally in the present study in group III in an effort to replace L-asparaginase with a natural product possessing immunomodulant activity. Virtually, addition of *Nigella saliva* seed significantly improved also the out come of treatment in our cases and proved a good anticancer agent as shown in Tables 1. The remission rate achieved in our group I is near to another study (20) but with no survival difference in relation to age, sex, ethnic groups and immunotype. We have observed a higher CR rate (above 90%) having better prognosis in younger patients. However, in sex and ethnic groups no difference was found but regarding immunotype, relatively a better outcome in L_I type disease. Larson *et al*. (21) have reported 85% CR rate with 7% refractory subjects while 8% died. In their study, CR in younger patients was higher (94%) below 30 years age i.e., near to our findings.

Bassan *et a!*. (22) have observed a lower CR rate (only 44%) with idarubicin (50 mg/m^2) and 89% and 62% with medium and normal doses (20 and 10 mg/m^2) therapy. Their CR was lower to ours which may be due to adult ALL subjects while ours were below 18 years. The relative difference between our and the above study was that they have worked on adult newly diagnosed, relapsed and chronic myloid leukemic transformed cases while we have included only ALL, freshly diagnosed cases. However, medium dose remission rate (89%) was just near to ours, although we had used daunorubicin in place of idarubicin. Earlier it has been reported that the development of side effects like pancreatitis, hyperbilirubinemia, diabetes mellitus, diarrhea , hypofibrinogene-mia were observed in more cases in the group treated additionally with L-asparaginase (22).

Similarly, in the present study, high incidence of these side effects was noted in group II. Decreased level of platelets (2 cases) and Hb (2 cases), along with an increased level of bleeding time (1 case), clotting time (3 cases) blood glucose (2 cases), blood urea (1 case) and total, direct and indirect bilirubin in 7, 2 and 3 cases, respectively. Also, high serum ALT (4 cases), ALP (3 cases) and amylase levels (2 cases) were observed in group II patients. This has clearly indicated that this treatment has produced serious adverse effects in the body during and after the treatment. However, such findings were not observed in other two groups (I and III).. Only one subject has shown high urea level in the group I. The group III treatment did not show such effects and thus appears to be the best regime among the tested ones for starting remission of ALL because of low side effects. The purpose of testing L-asparaginase and *Nigella saliva* seed was to compare their side effects and to possibly replace L-asparaginase with *Nigella sativa* seeds.

The relative %age of side effects developed in group II are similar to others studies (23, 24) who have reported CR rate of 63.1 and 64 6% in doxorubicin, vincristine, prednisolone and adramicin, doxorubicin, vincristine, prednisolone combination therapies, in adult ALL patients. These studies have differed from the present study perhaps due to age difference. Similarly, in an another study (25), CR was 75% after 35 days in patients who received L-asparaginase, vincristine, prednisolone and doxorubicin combination. This study, however, have reported less side effects as compared to ours. As far as ascertained, no scientific study was published on the efficacy of this treatment so far and, therefore, the use of *Nigella saliva* seeds in the treatment of acute lymphoblastic leukemia initiated by us was entirely a new endeavor and has opened new dimensions to be explored further for future research.

The mechanism of action of *Nigella sativa* seeds as an anticancer agent is still unknown but this may be due to its high thymoquinone contents as suggested by Badri *et al (*26) who have reported that benzopyrine-induced stomach cancer was reversed, along with increase in lipid peroxide and decrease in glutathione and its transferase activity in blood by thymoquinone in rats which is main content of volatile oil of the seeds and therefore, a powerful chemo-preventive agent in stomach tumors. They have also reported antioxidant and anti-inflammatory activities coupled with enhancement of detoxification processes by these seeds. El-Kadi *et al*. (27) have reported improvement of immune system with *Nigella saliva* seeds in experimental subjects. The use of seeds has already been recommended in their multimodality immunotherapy program for treatment of advanced cancers (28). From data discussed, it is conceivable; therefore, that *Nigella sativa* seeds could be used in treating acute lymphoblastic leukemia in children when given in combination with other cytotoxic drugs.

Table 1: Sex distribution and remission rate in ALL patients in different groups at start

Remission status	Males			Females			Total
	Group I	Group II	Group 111	Group 1	Group 11	Group III	
CR	8	8	8	8	8	8	48
Missing	3	1	4	-	2	-	10
NR / Died	I	I	-	1	-	I	04
Total	12	10	12	9	10	9	62

CR= Complete remission, PR= Partial remission, NR= No remission

Table 2: Remission status in different morphologic subtypes of 62 ALL patients at start

Remission status	L_1				L_2				L_3				Grand Totals
	Group 1	Group ll	Croup III	Total	Group I	Group I1	Group III	Total	Group I	Group II	Group III	Total	
CR	6	5	5	16	9	8	10	27	I	3	I	5	48
Missing	I	1	2	4	1	2	1	4	0	I	I	2	10
NR/ Died	0	0	0	0	I	0	0	I	I	I	I	3	4
Total	7	6	7	20	II	10	II	32	2	5	3	10	62

CR= Complete remission, PR= Partial remission, NR= No remission

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