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Evaluation of the effectiveness and safety of N-chromosome Royal Jelly on the number of peripheral white blood cells in patients with acute lymphoblastic leukemia

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Abstract
Objectives: This study aims to investigate the immune response blood cells following N-chromosome Royal Jelly (RJ) administration in patients with childhood acute lymphoblastic leukemia (ALL).
Methods: This single center before and after 12-weeks clinical trial was done in Ali-Asghar Children Hospital on 26 patients diagnosed with ALL during maintenance phase of chemotherapy. After collecting their demographics, patients received a starter dose of 2-g processed new natural N-chromosome RJ before breakfast and were followed up every 2 weeks, counting their peripheral white blood cells (WBCs), absolute neutrophil count (ANC), absolute lymphocyte count (ALC) by a blood cell count and differential analysis.
Results: A total of 26 patients were enrolled in this study (16 males and 10 females). Mean peripheral WBC count of total patients were raised significantly after administering N-RJ. Being 2510±1192 cells per cubic millimeter at the beginning, and then raised to 4549±1500 cells per cubic millimeter at the end of trial. ($p<0.005$). None of patients suffered from any adverse reaction during the trial.
There was a positive statistical relationship between total peripheral WBC, ANC, and ALC count and N-chromosome RJ increased dosage. Being more prominent at the beginning of trial ($p<0.001$) and the last 2 weeks of follow-ups. ($p<0.0005$)
Conclusion: This study has successfully demonstrated that N-chromosome RJ can be a promising immune-enhancing supplement in patients diagnosed with ALL in their complete remission and maintenance therapy time. In addition, it is a natural alternative for drugs like granulocyte colony-stimulating factor, but without those long-term adverse effects, we see in G-CSF drugs.
Keywords: Acute lymphoblastic leukemia, N-chromosome Royal Jelly, white blood cell

Introduction

Childhood acute lymphoblastic leukemia (ALL) is the most common cancer diagnosed among pediatrics, consisting nearly 25% of malignant disease in children.it is more common among males, with a peak incidence between 1 and 4 years of age.[1] Symptom of ALL are generally non-specific and numerous and include prolonged fever, petechia, bone pain, and palpable liver or spleen detected during physical examination.[2] The typical duration of chemotherapy treatment is usually 2–3 years, and consists of induction, consolidation, and maintenance therapy.[3] Most of the drugs used for treating ALL in children have several adverse effects, which can be mild or even fatal, so risk-directed stratification contributes to suppress relapse risk and avoid excess complication.[4] Granulocyte colony-stimulating factor (G-CSF) has been used to ameliorate drug induced myelosuppression.[5] Guidelines indicate the use of G-CSF whenever the chance of febrile neutropenia is up to 20% or more.[6] Several adverse effects including bone pain, arthralgia, myalgia, thrombocytopenia, epistaxis, cough, dyspnea, fever, nausea, back pain, acute respiratory distress syndrome, alveolar hemorrhage and hemoptysis, severe sickle cell crises in patients with sickle cell disorders, cutaneous vasculitis, sweet's syndrome, and osteoporosis have been reported following G-CSF administration.[7-11]

Royal Jelly (RJ) is a bee product secreted from the hypopharyngeal and mandibular glands of worker bees. There are many reports on pharmacological activities of RJ in experimental animals, but there are few about its antioxidative properties connected to aging. In addition, preliminary studies have showed that use of RJ as an immunomodulatory supplement can have numerous positive in vivo and in vitro effects.[12-13]

Nowadays, researchers have been studying RJ benefits on healing different types of disease including peptic ulcer, malignancies, and diabetes. The mixture of both workers jelly and RJ is called N-chromosome RJ, which have elucidated to have a significant healing feature to treat peptic ulcers.[14]

The present study was undertaken for the first time in the world to investigate the effect of fresh oral prescribed N-chromosome RJ, a new product of RJ on immune cell counts of patients with childhood ALL who were chemotherapeutically treated and were in complete remission and maintenance state. Various blood chemical analyses were conducted to count peripheral WBC, absolute neutrophil (ANC) and lymphocyte count (ALC) to analyze concurrent change in blood immune cells during the trial.

Materials and Methods

This single-center, randomized study was carried out on 26 patients who were previously diagnosed with ALL and were in complete remission and maintenance state of their chemotherapy treatment. Patients were visited in the pediatric oncology clinic of Ali Asghar Hospital in Tehran (Iran) between April 2020 and June 2020, after obtaining approval from our local ethics board and after receiving registry codes from the Iranian Registry of Clinical Trials (Registration code: IRCT) and after taking ethical consent and explaining different aspects of study, patients were enrolled in the trial.

Inclusion criteria were as follows:

1. Age less than 18-year-old.
2. Having the confirmed diagnosis of ALL.
3. All enrolled patients have been treated by IC-BFM2009,[1] in complete remission and in the maintenance phase of chemotherapy.
4. Approvement of clinical trial taken by ethical consent.
5. Not having a previous history of honey-related compound allergic reaction.

Patients who received G-CSF or other hormonal immune-targeted therapy were excluded from the trial. In addition, children with underlying disorders (such as Down syndrome) were excluded from the study. Demographic data including age, sex, type of malignancy and its stage, duration of treatment, and type of immunophenotyping were recorded for each patient. Also, a cardiac monitoring was done by a simple echocardiography by an expert pediatric cardiologist to rule out any cardiac background disease.

Processed new natural N-chromosome RJ is a homogenous component of honey used by queen bee due to its rich nutritional and immunological characteristics. It is yellowish in color with brown tinges and semi-soluble in water. N-chromosome RJ refers to the RJ produced by nurse bees for queen cells that have drone larvae grafted onto them. Study participants received a starting dose of 2 g processed new natural N-chromosome RJ before breakfast during their maintenance phase of chemotherapy and dosage were doubled according to their immune-cell counts and response. Each patient was followed up by taking a total CBC and cell differential every 2 weeks and assessment was done till the end of 12 weeks of treatment.

Data Analysis

Patients' information was entered into SPSS v23.0. Descriptive data were analyzed by descriptive tests. Parametric and non-parametric tests was employed and p-value less than 0.05 was considered as significant.

Ethical Considerations

Patient information was only available to the executor and the name of the patient remained confidential. Research team members were aware of the details of Helsinki statement about ethic principles in medical research and were strictly committed to follow them in this research study. This project was approved at the Ethics Committee of the University of Medical Sciences.

Results

This study is a single center pilot clinical trial. All patients were evaluated before treatment, and every 2 weeks lasting for 12 weeks after intervention. During each visit, a complete CBC and differential cell count analysis were done and N-chromosome RJ dosages were titrated according to immune-cell response in each patient.

This study included 26 patients diagnosed with ALL (16 males and 10 females) who were during maintenance phase of their chemotherapy protocol. The mean age of total patients was 5.78 years. Cellular immunophenotyping showed that 24 patients (92.3%) had B-cell ALL and the others (7.7%) were treated as T-cell ALL (Table 1).

None of patients had an allergic reaction, hypotension, or respiratory adverse effect after taking N-chromosome RJ and mean WBC count of total patients at the beginning of trial was 2510 ±1192 cells per cubic millimeter, while it raised to 4549±1500 cells per cubic millimeter at the end of trial.

There was significant statistical relationship between total peripheral WBC, ANC, and ALC count and N-chromosome RJ increased dosage. Being more prominent at the beginning of trial ($p<0.001$) and the last two weeks of follow-ups ($p<0.0005$) (Table 2).

There was a positive correlation among peripheral WBC, ANC, and TLC count during every 2 weeks, especially when the N-chromosome RJ was started (Before trial and during first 2 weeks) showing an increase in total number of cells ($p=0.0001$) (Figs. 1–3).

Discussion

During recent decades, the prognosis of childhood ALL has improved dramatically, nowadays, reaching a cure rate of almost 90%.[15] Chemotherapy is the mainstay treatment in patients diagnosed with ALL and it consisted of three different phase including induction, consolidation, and total remission and maintenance phase.[16] Chemotherapy-induced neutropenia is one of major dose limiting effects of chemotherapy, usually known as the most serious hematologic toxicity of chemotherapy,[17-18] thus clinicians have been using different type of drugs, trying to ameliorate neutrophil counts in patients who have undergone chemotherapy. For example, in our prior study, we have evaluated G-CSF positive effects on patients with ALL. However, administering G-CSF can cause various adverse effects in patients.[17,19] Since previous studies have successfully indicated that RJ can have anti-oxidative and immunomodulation effects.[12-13] In a study published by Kaftanoglu published in 1997, he and his colleagues have successfully indicated that using RJ in patients suffering from leukemia can increase the average peripheral WBC, neutrophil and lymphocyte.[12] In addition, Shirzad and colleagues in a study published in 2013 have concluded that use of RJ in syngeneic mice suffering from fibrosarcoma can significantly reduce tumor size ($p<0.05$).[20]

Our purpose in this study was to evaluate N-chromosome RJ effects on blood cell counts, especially lymphocyte and neutrophil absolute count and to see its potentials in patients diagnosed with ALL in complete remission and maintenance phase. None of patients who were included experienced an allergic reaction to administered N-chromosome RJ. Additionally, this study indicated that administration of N-chromosomal RJ can significantly increase peripheral WBC, ANC, and TLC in patients suffering from neutropenia ($p<0.0005$). More recently, the lipophilic fraction of RJ was reported to have extraordinary anti-proliferative activities in a neuroblastoma cell line (SH-SY5Y) compared with hydrophilic fraction.[21] In addition, this study also found that the biological activities in neuroblastoma cells were stronger than immortalized murine myoblasts and prostate cancer cells. Thus, we also agree with their opinion that the search for more effective and disease-specific fractions of RJ may be critical for improvements in the anti-cancer effects.

1 The Berlin-Frankfurt-Münster (BFM) 2009 protocol for ALL chemotherapy.

Table 1. **Hematological markers according to Royal Jelly dosage per day in patients with childhood ALL.**

Hematologic Marker	Time of trial	RJ dosage	Mean (SD)	Hematologic Marker	Time of trial	RJ dosage	Mean (SD)	Hematologic Marker	Time of trial	RJ dosage	Mean (SD)
WBC count	Before trial	qd	2553(1537)	ANC count	Before trial	qd	1097(647)	ALC count	Before trial	qd	670(363)
		BID	2590(797)			BID	1069(355)			BID	758(388)
		TID	2638(909)			TID	1104(380)			TID	650(182)
	Week 1	qd	3533(936)		Week 1	qd	1638(389)		Week 1	qd	1099(409)
		BID	3383(739)			BID	1550(353)			BID	949(335)
		TID	3604(1151)			TID	1611(563)			TID	897(298)
	Week 2	qd	3241(339)		Week 2	qd	1547(274)		Week 2	qd	862(286)
		BID	4240(1493)			BID	2095(766)			BID	1228(572)
		TID	4171(1248)			TID	1990(624)			TID	1051(307)
	Week 3	qd	3637(646)		Week 3	qd	1766(316)		Week 3	qd	991(298)
		BID	4640(1706)			BID	2458(961)			BID	1360(604)
		TID	4312(822)			TID	1996(537)			TID	1260(335)
	Week 4	qd	4205(593)		Week 4	qd	2368(267)		Week 4	qd	1281(437)
		BID	5520(1972)			BID	3142(1197)			BID	1627(717)
		TID	4225(1023)			TID	2343(471)			TID	1326(442)
	Week 5	qd	4322(742)		Week 5	qd	2660(362)		Week 5	qd	1268(477)
		BID	6557(2871)			BID	4150(1874)			BID	2097(921)
		TID	6188(762)			TID	3751(423)			TID	1970(279)
	Week 6	qd	4903(778)		Week 6	qd	3025(528)		Week 6	qd	1524(361)
		BID	7221(3048)			BID	4969(2130)			BID	2309(995)
		TID	8654(464)			TID	5719(213)			TID	2838(167)

qd: once daily; BID: twice daily; TID: Three times a day.

Table 2. **Clinical and demographic profile of children diagnosed with ALL.**

Demographics	Number (%)
Age (Mo.)	5.78
Sex 1. Male 2. Female	 16(61.5%) 10(38.5%)
ALL type 1) B-Cell 2) T-Cell	 24(92.3%) 2(7.7%)
Hypotension	None
Cardiac disease	None
Respiratory disease	None
Allergic reaction	None

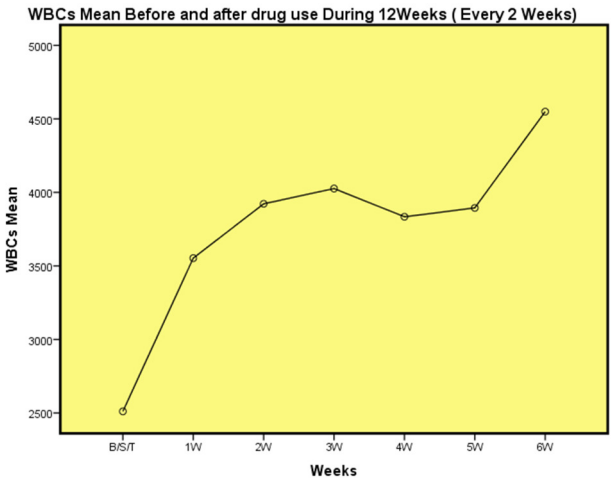

Fig. 1 **Whole blood cell count before and after administering N-chromosome Royal Jelly.**

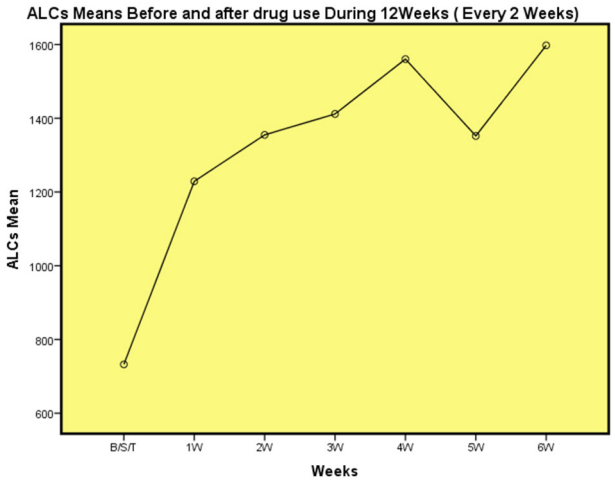

Fig. 2 **Absolute lymphocyte cell count before and after administering N-chromosome Royal Jelly.**

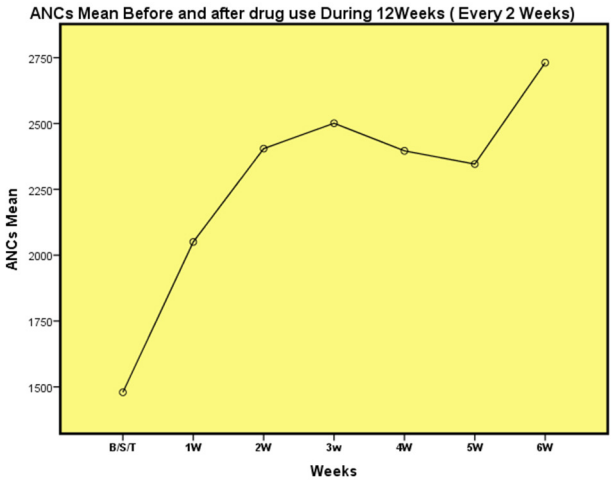

Fig. 3 **Absolute neutrophil cell count before and after administering N-chromosome Royal Jelly.**

Consistent with previous studies, we showed that administration of RJ can successfully increase immunological cell counts and it is an effective supplement which can be effectively administered without any adverse effect in comparison with other drugs such as G-CSF.

Since this study was done in a private oncologic clinic, one limitation of our study could be a small sample size. Secondly, we couldn't evaluate dose–response effect of N-chromosomal RJ effect in patients. Future investigation is warranted to further characterize these findings and their relevance to prognosis in patients with ALL.

Conclusion

To our knowledge, this is the first pilot study to suggest that use of N-chromosomal RJ supplement can significantly increase immune cells count in patients that were in total remission and maintenance phase during chemotherapy–chromosome RJ should be considered as an effective alternative supplement in comparison with G-CSF.

Clinical Trial Registration

The study was registered in the Iranian Clinical Trial Registry Center (http://www.irct.ir) with the registration code: IRCT20190106042260N1

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Conflict of Interest

There is no contradiction in the article.

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