Original Article

The Possible Association between Tumor Necrosis Factor Alpha C-850T Polymorphism and Childhood Acute Lymphoblastic Leukemia

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ABSTRACT

KEY WORDS: acute lymphoblastic leukemia, hematological malignancies, leukemia

Objective: To study the possible association between tumor necrosis factor alpha C-850T polymorphism and childhood acute lymphoblastic leukemia (ALL) in Khartoum, Sudan

Design: Prospective analytical case-control

Setting: Medical Laboratory Research Center (MLRC), Khartoum State Teaching Hospital (KSTH), Sudan

Subjects: Sixty-six patients diagnosed with ALL and forty-three apparently healthy adult controls admitted from October 2003 to January 2008

Intervention: Measurement of total white blood cell count, hemoglobin level and genotyping using Sysmex automated analyzer and PCR-amplified

Main Outcome Measure: Analysis of data obtained from the MLRC of the KSTH, Sudan, to determine allele frequency

Results: There was a statistically non-significantdecrease in the relative frequency of the TNF〈 -850T allele detected in the ALL group during the study period (p = 0.123). In children with B-ALL a more pronounced but still marginally significant(p=0.042)decreasewasfound. The odds ratio (OR) for the presence of TNF〈 -850T allele (CT and TT) was 0.653 (95% CI = 0.327-1.302). The corresponding value for B-ALL, was 0.548 (95% CI = 0. 256 -1.174).

Conclusion: The statistically non-significantassociationsof TNF〈 - 850T polymorphism suggests the need to investigate the risk for childhood ALL or the presence of fever, anemia, leukocytosis and leukopenia at diagnosis.

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INTRODUCTION

Tumor necrosis factor alpha (TNF〈), a 17 kilo Dalton (KDa) protein (a cleavage product of a 29 KDa membrane-associated protein) encoded by gene stationed on chromosome 6 near the major histocompatiblity complex, is capable of functioning as a direct inhibitor of progenitor cell growth[1]. TNF〈 is a potent immunomediator and proinflammatorycytokine,implicatedinanumberofdisorders[1]. As it promotes the growth of lymphoid cells and influencestheprognosisandresponsetotherapy of patients with cancer it presents itself as a good candidate for an association with hematologic malignancies and their clinical manifestations[2]. Higher levels of serum TNF〈 have been detected, at diagnosis, in children with malignancies, including acute lymphoblastic leukemia (ALL)[3]. Also, higher levels of TNF〈 have been associated with febrile episodes at diagnosis in children with acute leukemia[4]. Polymorphisms in the promoter region of the TNF〈 gene are known to affect plasma levels of the cytokine, and TNF〈 high producing alleles were associated with disease progression on some forms of hematological malignancies [2] but not in childhood ALL[5].

The C □ T substitution at position – 850 (C-85OT) gives rise to a polymorphism recently identifiedinthe promoter region of the human TNF〈 gene[6,7]. The polymorphism lies very close to two other, previously described polymorphisms, reported to affect the transcriptional activity of the TNF〈 gene, namely TNF〈 C-857T and TNF〈 C-863Q, as opposed to other TNF〈 promoter region polymorphisms[1]. To the best of our knowledge, no recent study has examined, so far, the possibility of an association of TNF〈 C-850T with any type of hematologic malignancy. On the other hand, the fact that TNF〈 C-850T was shown to affect the risk for vascular dementia in at least one population[8] argues in favor of a functional significanceforthispolymorphism.

Therefore, the main aim of this study was to analyze the distribution of the TNF〈 C-850T polymorphism in children with ALL, as well as in healthy controls in Khartoum, Sudan, and to examine its possible

association with clinical and laboratory findingsatdiagnosis,suchasfever,anemia, leucocytosis and leucopenia.

PATIENTS AND METHODS

This is a prospective analytical case-control based study conducted in Khartoum State Teaching Hospital (KSTH), Sudan during the period from October 2003 to January 2008 to determine the possible association between TNF〈 C-850T polymorphism and childhood ALL in the Sudanese population.

A total of 66 patients were analyzed. All patients had been diagnosed as having ALL (36 boys, 30 girls). Fifty-two out of these patients (30 boys, 22 girls), had been diagnosed as having B - cells ALL and the rest had pre B - ALL and T- ALL. All patients were under treatment. The study also included 43 apparently healthy adult individuals (20 male, 23 female) as control group. Both patients and controls were less than 15 years of age and were Sudanese nationals.

This study was approved by the local ethical hospital committee.

A structured questionnaire consisting of items including demographic data, family history and laboratory investigations was administered to all patients. An informed consent was obtained before collection of blood samples. Blood sample was drawn using a 20 or 21 G needle with limited occlusion of the arm by a tourniquet. The blood was added to the anticoagulant at a ratio of 2.4 ml of blood to 4.2 mg of EDTA (ethylenediamine tetra-acetic acid) and gently mixed. This sample was used for automated platelet count. The clinical analysis (including presence or absence of fever) and laboratory analysis (total white blood cells - TWBCs, hemoglobin (Hb) level and genotyping) was done for all patients at the time of diagnosis.

Laboratory Methods

Laboratory analysis was done in all patients and controls by measuring TWBCs, Hb level and genotyping analysis.

TWBCs and Hb level was measured using Sysmex automated analyzer; the genotyping was accomplished essentially brieflyintheregionof the TNF〈 promoter containing the C-850T polymorphism, was PCR-amplified,usingasprimersthe oligonucleotides: AAGTCGAGTATGGGGACCCCCGTAA (forward) and CCCCAGTGTGTGGCCATATCTTCTT (reverse). The amplificationconditionswere 94 ºC for 3 min, followed by 40 cycles of 94 ºC for 30s, 68 ºC for 30s and 72 ºC for 1 min and a finalextension step of 73 ºC for 10 min. The amplifiedDNAwas then subjected to Hind 11 digestion at 37 ºC for 4h, and subsequently electrophoretically separated in a 3.5% agarose gel. Under these conditions, the presence of the C allele is revealed by a 108 bp band, whereas that of the T allele by a 133 bp band.

Statistical analysis

Genotype and allele frequency distributions were compared between cases and controls with the X_2 test of independence. Odds ratios (OR) were calculated with a 95% confidenceinterval(CI).TheFisher'sexacttest was used to examine the association between carriage of the TNF〈 –850T allele and clinical and laboratory findingsatdiagnosis.

RESULTS

The observed genotype and allele frequencies for patient and control groups are shown in Table 1. An insignificant(p=0.123)decreaseintherelativefrequency of the TNF〈 – 850T allele was detected in the ALL group compared to the control group. When the patient group was limited to those children diagnosed with B - ALL (n = 52 out of 66) the effect was more pronounced, but still only marginally significant(p=0,042,andwithYate'scorrectionp=0.1, p = 0.05). The calculated OR for developing ALL in the presence of the TNF〈 – 850T allele (CT and TT genotypes) was 0.653 (95% CI = 0.327-1.302). The corresponding value for B-ALL was OR = 0.548 (95% CI = 0.256-1,174).

Stratificationaccordingtosexdidnot affect the results. It seems likely that the TNF〈 C-850T polymorphism was not directly associated with childhood ALL, and that the observed under- representation of the TT genotype in the case samples was most likely due to a chance event. The distribution of the TNF〈 –850T allele among ALL

Group
n
Genotypes, n
pa
Alleles, n
p-value
CC
CT
TT
C
T

Control
43
23
17
13
122
52
Pre-B and T-ALL
14
9
3
2
0.385
90
26
0.123
B-ALL
52
33
16
3
0.171
72
16
0.042

Table 1: Genotype and allele frequencies of the TNF⟨ C-850 polymorphism in ALL children and controls

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patients, according to fever, anemia, leukocytosis or leukopenia at diagnosis, is shown in Table 2. No significantassociation(p=1.000,0.338,0.335and 0.119 respectively) has emerged from these comparisons. Limiting the patient group to B-ALL only, did not affect this result.

DISCUSSION

The results of this study did not show any direct effect of the TNF⟨ C-850T polymorphism either on the children acquiring ALL or on the occurrence of particular clinical and laboratory characteristics in the event of a positive diagnosis. However, two points deserve some comment. First, it could be argued that the rather small size of our study group limited the statistical power of this study. However, the relatively high prevalence of the TNF⟨-850T allele as well as the lack of a statistically significantdifference (p = 0.042) between the entire ALL group and the B-ALL subgroup support the validity of the results. Second, the choice of the control group was made solely to exclude the possibility that any of these individuals would have developed childhood ALL. Obviously, we could not exclude the presence of other pathologies in which TNF⟨ may be involved. This may represent a source of bias in this study. Finally, it should be taken into consideration that these TNF polymorphisms are found in a region of great polymorphic variation in linkage disequilibrium with the human leucocytes antigen (HLA) genes and with each other. Because of differences in the distribution of HLA alleles, one might expect variation in associations between TNF⟨ polymorphisms and various conditions, in different geographical areas. Dissecting out a primary association with TNF⟨ might not be an easy task. It is worth noting that in a recently conducted study, another polymorphism in the TNF locus (G-308A) was reported to increase the protein levels of TNF. This was shown to influencethe relapse in (prednisone poor responders) ALL patients. Even though no other effect was detected in any ALL subgroup[9] additional studies should be undertaken in order to examine the effect of TNF C-850T in various ALL subgroup, and in different ethnic groups, especially since the frequency of this particular polymorphism has been shown to differ considerably among Sudanese population [6, 9-11].

CONCLUSION

The TNF polymorphism were found in a region of great polymorphic variation in linkage disequilibrium with the HLA genes and with each other. Because of differences in the distribution of HLA alleles, one might expect variation in associations between TNF⟨ polymorphisms and ALL, in different geographical areas. Dissecting out a primary association with TNF⟨ might not be an easy task. In conclusion, TNF⟨ –850T polymorphism was not directly associated with childhood ALL, and no statistically significantassociationshaveemerged between this polymorphism and either the childhood ALL or presence of fever, anemia, leucocytosis and leucopenia at diagnosis.

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Anemia - Hb < 9 g/dl, Leucocytosis - WBC > 14000/mm3, Leucopenia - WBC < 6000/mm3, d - CT and TT genotypes, e - CC genotypes,
F - Fisher test

Table 2: Relationship between TNF(– 850T allele carrier status and clinical and laboratory findingsatdiagnosis,inALLchildren

Allele carrier status	**Fever**	**Anemia**	**leukocytosis**	**Leucopenia**
+				
-				
+				
-				
+				
-				
+				
-				
TNF(–850T +d	9	7	10	4
	8	6	3	11
TNF(–850T -e	20	14	15	13
	14	20	16	18
SignificanceF(p-value)	1.000	0.338	0.335	0.119

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