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ORIGINAL ARTICLE

The Expression of Interferon Gamma (IFN-γ) and Interleukin 6 (IL6) in Patients with Acute Lymphoblastic Leukemia (ALL)

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Abstract

Interferon gamma (IFN-γ) and interleukin 6 (IL-6) are including the most important cytokines which have been associated with the biological behavioral and immune responses in malignancies. Based on the critical roles which these two cytokines play against tumor cells, the present study was aimed to investigate the genes expression level of IL6 and IFN-γ in patients with Acute Lymphoblastic Leukemia and compare with normal controls. Fifty-two patients with ALL and 13 healthy volunteer were under studied. The peripheral blood mononuclear cells of all patients and normal controls were separated by ficoll. The expression of interferon gamma and interleukin 6 genes were determined by RQ-PCR. Finally all data were analyzes using T student, one way ANOVA and Mann-Whitney tests were use to analyze all samples data. Our finding showed that the level of IFN-γ gene expression was significant decreased in patients with All as compared with healthy controls (83 change fold, $p < 0.0001$). The level of IL-6 Gene expression was not changeable in B-ALL patients as compared with healthy control ($p = 0.4$), but in T-ALL patients, was significantly reduced ($p < 0.01$). The results of present study indicated that IFN-γ gene expression reduced in ALL patients. It provides a valuable insight that immune system may disrupted in patients with ALL, which cause tumor cells escape from immune surveillance.

Keywords ALL · IFN-γ · IL-6

Introduction

Acute lymphoblastic leukemia (ALL), the most frequent cancer in children [1], is a malignant disease that arises from the clonal expansion of lymphoid progenitors that have undergone neoplastic transformation at distinct stages of differentiation [2]. Several genetics and epigenetics alterations have been recognized to have a tight correlation with the pathogenesis of this malignancies, which among these factors cytokines are the most important ones. It has been suggested that cytokines, a low molecular weight glycoproteins, could implicate the immune responses and determine the behavior of malignant disorders, in particular ALL, toward cancer treatment [1, 2].

Interferon- γ (IFN-γ) and Interleukin 6 (IL-6) are two important cytokines which play the critical roles against tumor cells. IFN-γ produces by natural killer cells and T- helper cells which are involving in both innate and adaptive immune responses [3]. The potent anti-tumor effect of this cytokine has been reported in several investigations in different tumor cell lines. Moreover, kholoussi et al., suggested that the plasma level of IFN-γ has significantly increased in patients with ALL before any treatment [4]. As another inflammatory cytokine in many chronic disorders, IL-6 has a critical role in promoting cancer progression and severity [5, 6].

Several studies have been reported that the concentration of IL-6 was higher in leukemic patients than healthy counterparts, suggesting that this cytokine can be considered as a prognostic factor in ALL [7]. Given to the important roles of IL-6 and IFN-γ in malignancies, in this study we investigated the gene expression of these cytokines and monitored the

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correlation between their expression and clinical condition in ALL patients.

Materials and Method

Patients

During the years 2014 to 2016, 52 patients diagnosed with ALL at Mofid and Taleghani Hospitals (Tehran) coupled with 13 healthy volunteers as a control group were chosen for this study. The protocol of this study was approved by hospitals research Ethics committee (IR.SBMU.RETECH. REC.1393.163) and informed consent obtained from every participants in accordance with the Deceleration of Helsinki.

Sample Preparation

The samples provided from both Bone Marrow and Peripheral Blood of 52 patients (Bone Marrow: 38 Peripheral Blood: 14) and 13 healthy controls. Demographic characteristics of patients (age, gender, type of samples) are summarized in Table 1. WBC of Bone marrow and peripheral blood were differentiated. The phenotype of blasts were determined using FACS (ABI, USA). Moreover, cytological variants of hematological malignancies were analyzed according to new WHO classification. Mononuclear cells from all samples were isolated using Ficoll density gradient (Behringer, Germany).

Table 1 Demographic characteristics of patients

	Variable	Study group ($n = 52$)
Age	<16 yr	20
Age	> 16 yr	32
Gender	Male	30
Gender	Female	22
	ALL Subtypes	–
Immunophenotype	Pro-B cell	4
	Early Pre-B cell	11
	Pre B cell	14
	mature Pre B cell	14
	T cell	9
	t(12, 21)	6
Chromosome abnormally	t(9,22)	5
	t(1, 19)	3
	t(4, 11)	1
	No translocation	37

RNA Extraction and cDNA Snthesis

Total RNA from mononuclear cells of patients and controls were extracted using a High Pure RNA Isolation Kit according to the manufacturer's recommendation kit (Qiagen, Germany). The quantity of RNA samples was assessed spectrophotometrically using Nanodrop (Thermo scientific, USA) and qualified the RNA by gel electrophoresis (Invitrogen, USA). The reverse transcription (RT) reaction was performed according to the fermentase kit (thermo scientific – USA).

Quantitative Real-Time PCR

Alteration in the mRNA expression level of IFN-γ and IL-6 were assessed by real-time PCR, performing with a light cycler instrument (ABI, USA) using SYBR Premix Ex Taq technology (Amplicon, Denmark). To do so, real-time PCR assay was performed in a ultimate volume 15 μl of reaction mixture containing: 7.5 μl master mix (Amplicon, Denmark), 6.8 μl nuclease free water (Sincolon, Iran), 1.2 μl forward and reverse primer (Takapou zist, Iran), 1.5 μl cDNA (1.10) was amplified in thermal cycler. Cycler conditions were respectively: initial denaturation at 95 °C for 20 s followed by 62 °C for 15 s to annealing and final extension at 72 °C for 20 s. Melting curves were analyzed to verify single PCR product of each primer. ABL gene was amplified as an internal control. The fold change in the expression of each mRNA were calculated using $2^{-\Delta\Delta Ct}$ relative expression formula. The sequences of the primers for IL-6 and IFN-γ were summarized in Table 2.

Statistical Analysis

All experiments were performed in triplicate and statistical analyses were performed with SPSS software (version 21). *t*-student, one way ANOVA and Mann-Whitney test were used to analyze all data. Pearson correlation test was used to evaluate the correlation of results. A probability level of $P < 0.05$ was considered statistically significant.

Results

We investigated the mRNA expression level of IFN-γ gene in both ALL patients and healthy controls using RQ-PCR analysis. Our results showed that expression level of IFN-γ significantly decreased in patients in comparison with control group ($P \leq 0.001$). As depicted in Fig. 1, the gene expression of patients were 83-fold lower than the healthy volunteers. Moreover, to examine whether the gene expression level of IL-6 was changed in ALL patients in similar pattern to IFN-γ, we determined the mRNA expression level of this gene using RQ-PCR. As shown in Fig. 2, there was no remarkable

Table 2 Gene name, primer sequence, amplified fragment length for all three genes

Gene	Forward primer (5′-3′)	Reverse primer (5′ − 3)	Size (bp)
IL-6	GACAACTCATCTCATTCTGC G	TAACAACAACAATC TGAGGTG	21
INF-γ	AAGTGATGGCTGAACTGTCG	GCAGGCAGGACAACCATTAC	20
ABL1	AGTCTCAGGATGCAGGTGCT	TAGGCTGGGGCTTTTTGTAA	20

difference in the mRNA expression level of IL-6 between ALL patients and healthy counterparts.

To find whether there is a correlation between IFN-γ and IL6 expression and gender, the alteration in the mRNA level of these two cytokines were examined between male and female groups according to Mann-Whitney test. The results showed that the expression level of aforementioned genes was similar between both male and female ALL patients. Additionally, the comparison of IFN-γ and IL6 expression according to the two aging groups (less and more than 16 years old) revealed that there is no significant correlation between genes expression and age. To investigate the association between ALL classification and the expression of IFN-γ and IL-6, we classified our samples into five groups; T-ALL, B-ALL, pre B-ALL, Early pre B-ALL, Pro B-ALL, using flow cytometric analysis. The results of ANOVA analysis showed that the alteration in the genes expression levels were independent of different types of ALL (Fig. 3).

Demonstrating the gene expression in ALL patients who had cytogenetically abnormal chromosome in comparison with patients without any abnormality showed that while there was no difference in the expression of IFN-γ between two groups, the mRNA expression level of IL-6 was lower in patients with positive translocation than those with negative translocation ($p = 0.047$) (Fig. 4). Moreover, as indicated in Fig. 5, although the expression level of IL-6 was quite similar between B-ALL patients and the healthy volunteers ($P = 0.4$), the mRNA level of this gene was significantly lower in T-ALL patients as compared to the control group ($P = 0.001$) (Fig. 5).

Discussion

IL-6, producing by different cell types, including lymphocytes, monocytes, and macrophages, is a multifunctional cytokine which play a fundamental role not only in regulating diverse cellular processes but also in the pathogenesis of a variety of diseases such as myeloma, Ki-positive large-cell anaplastic lymphoma, immunoblastic lymphoma, small lymphocytic lymphoma and acute leukemia [8]. On the other hand, interferon (IFN) family, basically involved in host defense against viruses and tumors, could bind to their receptors and through stimulation of jack/stat signaling pathways [9, 10], regulates the expression of diverse genes and protein production participating in inducing cell death in malignant cells, such as acute leukemia [4, 11, 12]. In the present study,

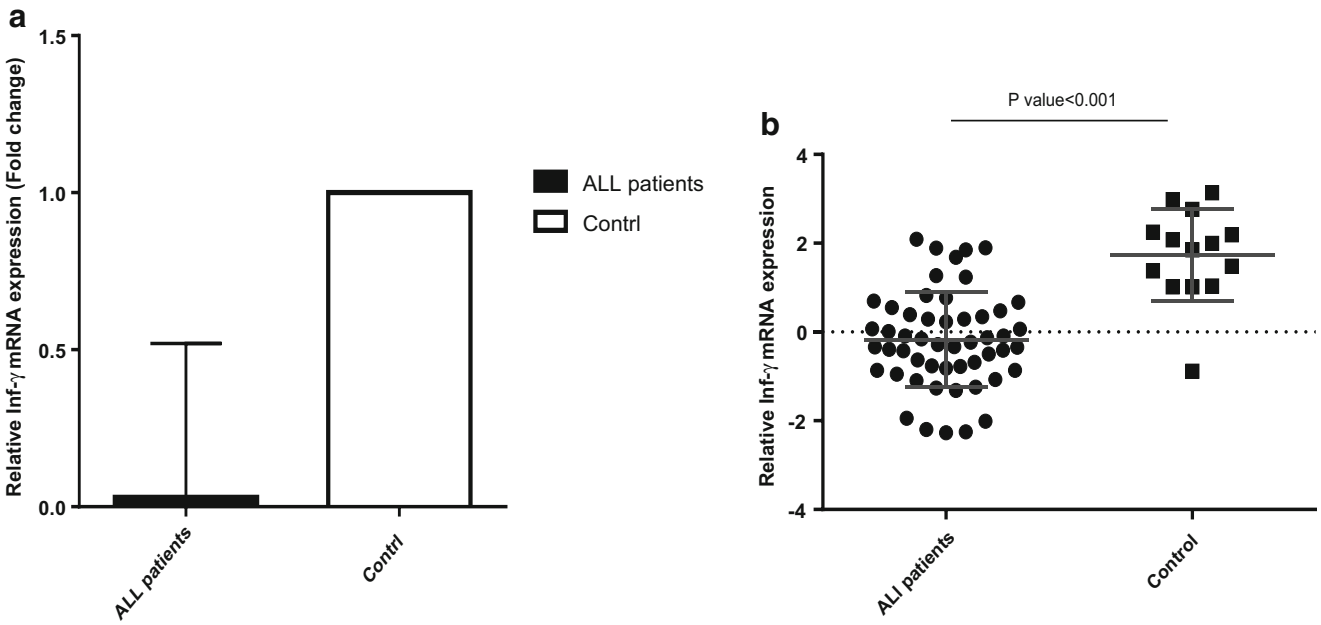

Fig. 1 **The IFN-γ gene expression in ALL. a** The expression level of IFN-γ decreased by 83-fold in ALL patients compared with controls ($P < 0.001$). **b** The comparison of IFN-γ expression between patients and controls

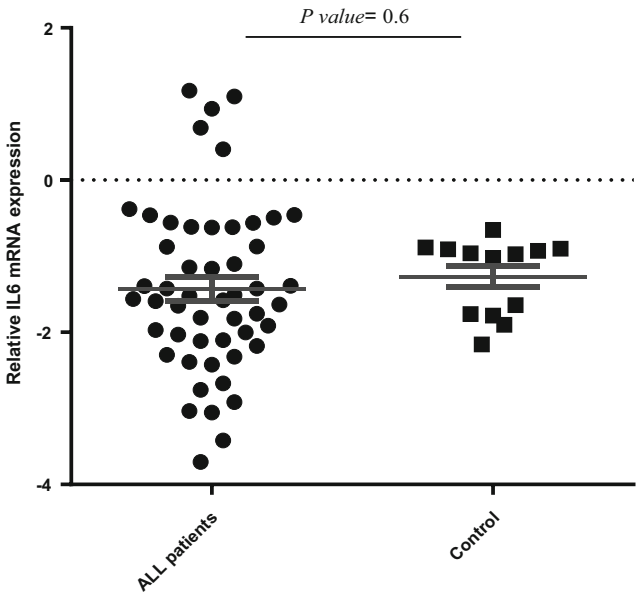

Fig. 2 The mRNA expression level of IL-6 in patients and control groups. There was no significant difference in the expression of IL-6 between patients and controls

we investigated the expression level of IL-6 and IFN-γ in ALL patients by real-time PCR. Moreover, we investigated whether there is a correlation between the expression of these cytokines with age, gender, immunophenotype, and blast count and chromosome abnormalities of ALL patients.

Our results showed that the mRNA expression level of IFN-γ is significantly decreased in ALL patients as compared to controls. This finding was in agreement with YIN et al. [13] and Park et al., [14] studies, suggesting that the intracellular level of IFN-γ was statistically lower in ALL patients. Additionally, they showed that the level of IFN-γ returned to normal when the patients were in remission stage. This finding was also evident in another study conducted by Zhang et al., which indicated that the level of IFN-γ returned to normal after complete remission [15]. Moreover, it is reported that IFN induces remissions in a variety of neoplastic disease [16], and plays an important role in the regulation of proliferation and differentiation of malignancies cells [17]. It is believed that the reduction in the expression of IFN may participate in tumorigenesis process. In this study, we did not find any significant correlation between IFN-γ gene expression and different clinical criteria, such as age, gender, ALL subtypes, blast count and chromosomal translocations, suggesting that probably the decreased gene expression is a result of common mechanism, such as a decreased sensitivity to IFN or an inability to produce IFN [18]. In a study, Wu et al. compared IFN-γ genes expression in ALL patients with or without t(12;21) translocation and they could not find any valuable changes between two groups [19].

Investigating the expression level of IL-6 in ALL patients, we did not find any significant different between the expression of this gene in ALL patients and control group. Although there was no correlation between the expression of this cytokine with age, gender, blast count, the expression of IL-6 was significantly lower in patients with chromosome translocation. We also find out that the mean level of IL-6 gene expression in patients with T-ALL were reduced. Inconsistent with our results, Morales-Rojas et al. showed a higher level of IL-6 in the blood and saliva of patients with acute lymphoblastic

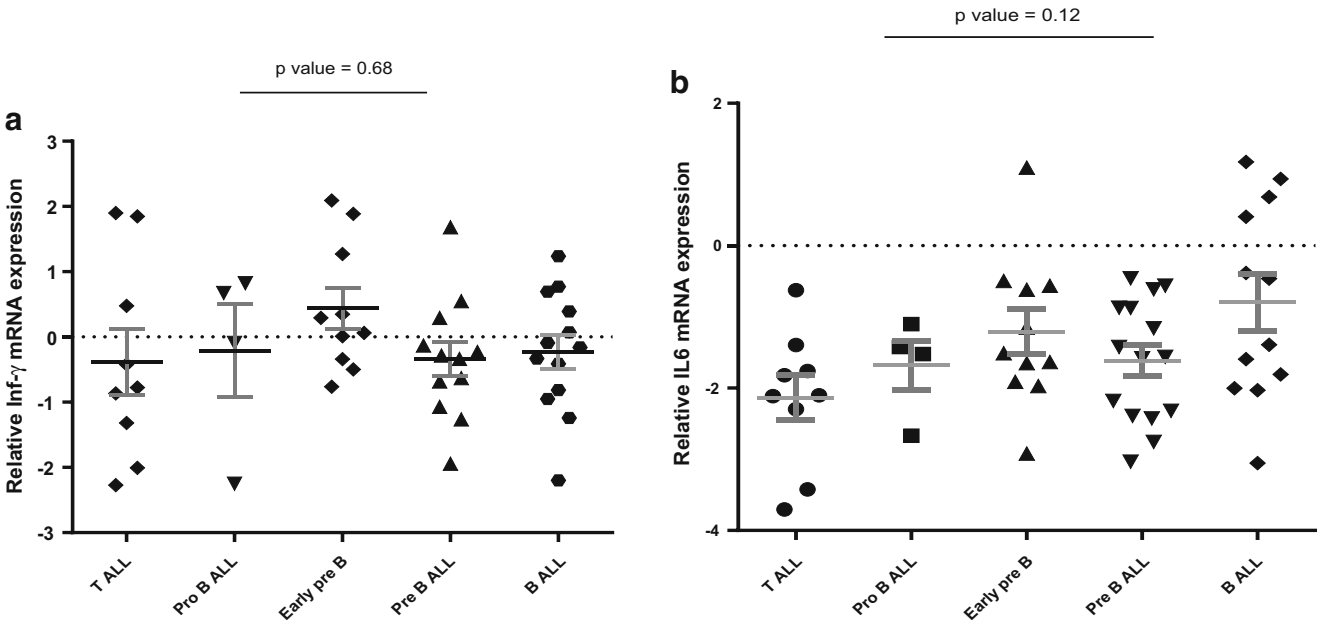

Fig. 3 The differences between the gene expression of IFN-γ (a) and IL6 (b) in ALL subtypes (T-ALL, B-ALL, pre B-ALL, Early pre B-ALL, Pro B-ALL). The results show that there was no correlation between the mean level of both cytokines and ALL subtypes. However, only in T-ALL, the expression of IL-6 was decreased in comparison with other subtypes

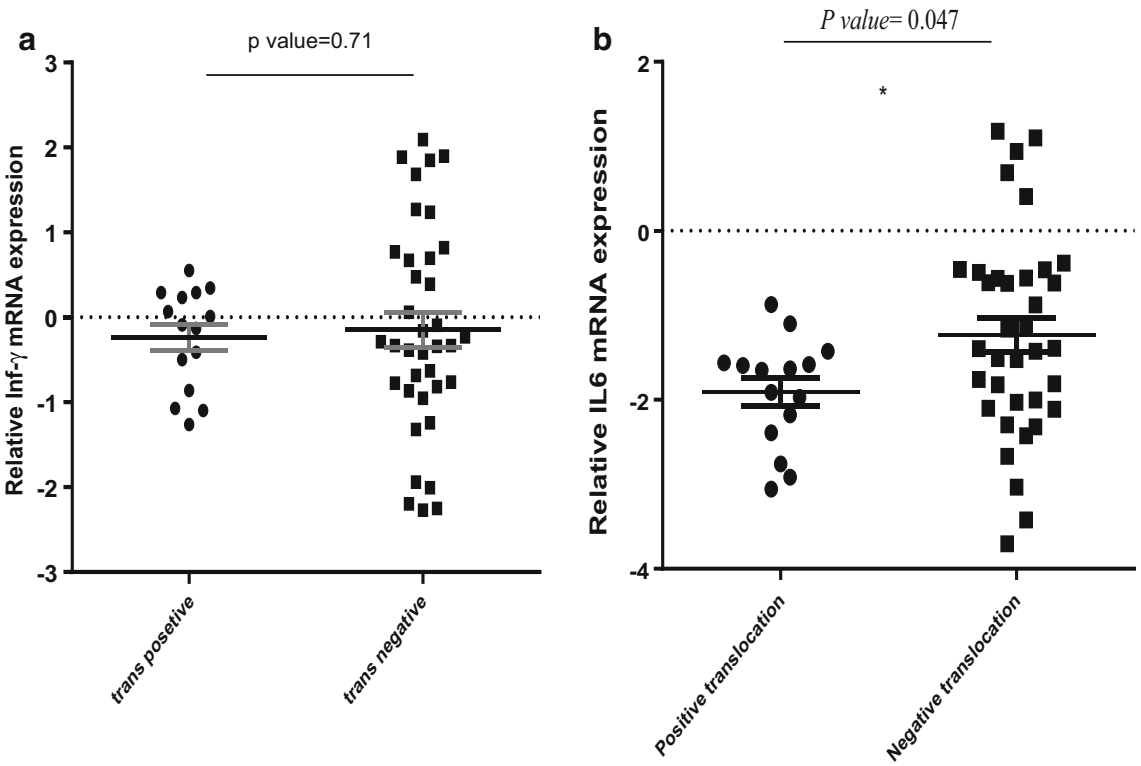

Fig. 4 The level of gene expression of IFN-γ and IL6 in two groups of patients with and without chromosome translocation. Although there was no significant difference in the expression of IFN-γ between two groups, the IL-6 expression level was remarkably lower in patients with positive translocation than those with negative translocation ($P < 0.05$)

leukemia [20]. Luo et al. also declared that the activity of IL-6 was significantly elevated in sera of patients with ALL and AML. Their results indicated that IL-6 can promote the proliferation of leukemia cells [21], suggesting that the abnormal level of IL-6 in patients with acute leukemia is probably related to the pathogenesis of disease. Several studies also indicated that the plasma level of IL-6 was associated with patient survival and event-free survival and low level of IL-6 represents favorable prognostic factors for patients [22, 23]. We suggest that further studies are needed to explore the effects of cancer treatments, in particularly immunotherapy, on the expression of these cytokines. Also further investigations for evaluating the expression of the receptors of these cytokines at the protein level could more precisely highlight their role in signaling pathways involved in tumorigenesis.

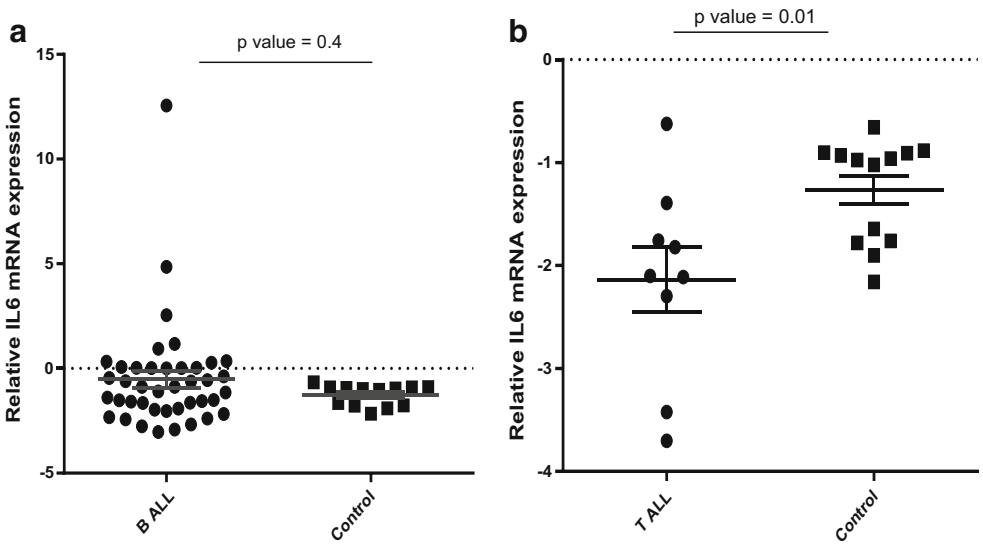

Fig. 5 The gene expression of IL-6 in patients with B-ALL and T-ALL. While the level of IL-6 expression was quite similar between B-ALL patients and healthy controls, this expression level was significantly lower in T-ALL patients as compared to the control group ($P < 0.01$)

Conclusion

The results of the present study showed a significant reduction in the intracellular level of IFN-γ, suggesting that immune system is disrupted in ALL patients, which could further aid tumor cells to escape from immune surveillance. It is proposed that probably the detection of IFN-γ can be used as the markers for monitoring the response to treatment in ALL patients.

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Compliance with Ethical Standards

Declaration of Conflicting Interests The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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