Study relationship between the human T lymphotropic virus infection and CML, AML Iraqi patients

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Abstract
leukemia is linked with many disease because of the interruptions in patients immunity and its defect mechanisms against the foreign pathogens. Although the percentage of infected leukemia patients with HTLV is rare but its very important to diagnose this virus especially in leukemia patients. the severity and treatment of HTLV is depend on the viral load and infection stage of virus.
Keyword: HTLV, *pol* gene, leukemia, CML, AML,

1. Introduction

Human Lymphocyte T Virus (HLTV) is one of viruses which involve in human lymphocytes infection and other components of human blood causing lymphoma and leukemia [1,2}. this virus has two types, the first type called (HTLV-1) while the second type called (HTLV-2). The genetic material of virus is RNA which is single strand [3].
it was found that approximately 10 to 20 million of human are infected with human T lymphocyte virus all over the world [4,5]. It was found that 0.25 % to 2 % of the infected patients will get another disorder or disease in companied with HTLV such as neurologic disease and lymph disorders and others. even though the virus percentage found is rare [6], but it cause a serous problems when cause an infection for human especially the low immunity or patient with auto immune diseases [7].

2. Material and Methods

One hundred and thirty leukemia patients were considered in this study 73 of these patients were CML, 57 were AML. Female samples were 80 while male samples 50. A sample of two ml blood was taken from every patient and DNA extraction were done using Gene aid DNA kit. Finally obtained DNA in a volume 50-100 µl dissolved in TE buffer in Eppendorf tubes and these tubes were then kept at 20-C°.

Detection of HTLV by pol gene specific primers

PCR used for determine pol gene to conform the identification of the HTLV, according to Cinna Gen Company the primers synthesized shown in (Table 1).

Table (1): The sequence and concentration of forward and reverse primers of pol gene.[8]

Primer type	Primers Sequence	Concentration in picomole	Product size
Pol forward	GTG GTG GAT TTG CCA TCG GGT TTT	30262.27	117 bp
Pol reverse	GTA CTT TAC TGA CAA ACC CGA CCT AC	35265.50	117bp

PCR reaction was conducted in master mix tube containing lypholyzied master mix table (2), with 20µl of reaction mixture containing, 1 µl of forward primer, 1 µl of reverse primer, template of DNA 5 µl and 13 µl of D.W. (Table 3).

Table (2);-AccuPower® PCR Premix content.

Reaction size	Component
1.5 µM	MgCl2
250 µM	dNTPs
1 U	Taq DNA polymerase
30 µM	Kcl
10 µM	Tris-HCL

Table (3): - The mixture of conventional PCR working solution for detection of, pol gene to diagnose the HTLV infections

Working solution	µl
Water	13 µl
Forward primer	1 µl
Reverse primer	1 µl
DNA	5 µl
Final volume	20 µl

Amplification was conducted using a master cycler Eppendorf programmed with 45 cycle consist of three minutes Initial denaturation at 95°C, then five second for denaturation at 95°C, followed by annealing at 60°C for 30 second, then extension at 72°C for 30 second and finally Extension at 72°C for five minute[9] .

RNA Extraction

The 130 blood samples of leukemia patients were taken. RNA of these samples was extracted by using RNA extraction kit (Geneaid extraction kit),

Gene expression

It was used four set of primers in this study, two primers are forward and reverse and another two primer are reference gene . cDNA was obtained from RNA using Genaid specific kit.
The expression of this gene was then calculated.

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3. Results and Discussion

This study involved 130 leukemia patients divided in to 73(56.15%) patients with CML and 57(43.84%) AML (fig 1) .
The age of patients was ranged from 15-71 years, among 130 patients the number of male is 50 (38.46%) involved 18 (36%) AML and 32 (64%) CML ,while female number was 80 (61.53%) involved 39 (48.75%) AML and 41 (51.25%) CML (fig 2).

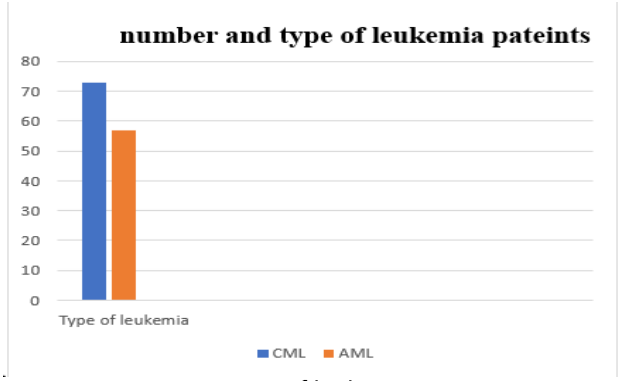

Figure 1: type of leukemia patients

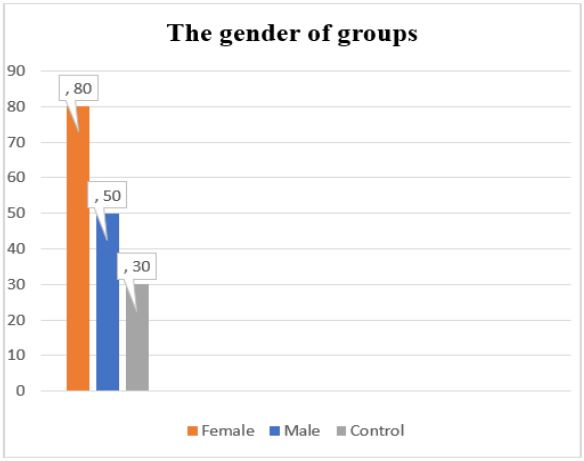

Figure 2: the gender of groups

From this study the result of PCR reaction showed that it was only 2 of leukemia patients infected with HTLV in a prevalence 1.53% (fig 3). Both of infected patients with HTLV were chronic myeloid leukemia (CML)

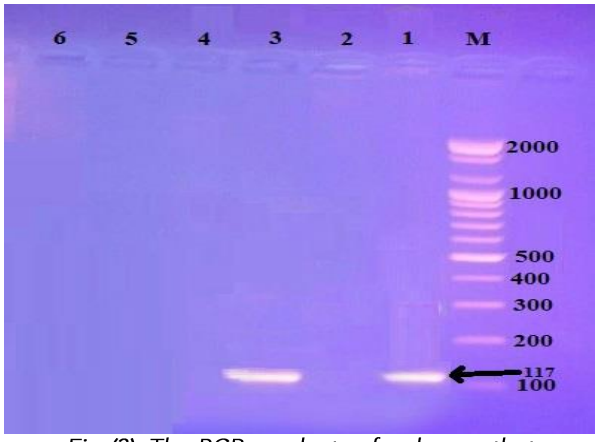

Fig (3): The PCR products of pol gene that electrophoresis in agarose gel with concentration 2% and under voltage 5 volt to each 1 cm

M: 100 bp molecular ladder; well (1 and 3) positive product of 117 bp for *pol* gene, well (2,5) were negative , well 6 is negative control.

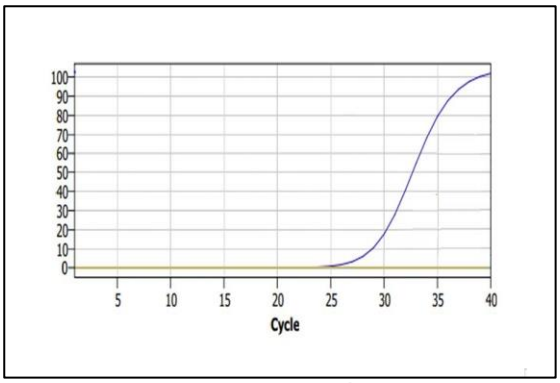

Fig (4): The RT-PCR of pol gene

Many studies conducted in the world to detect HTLV and its association with other disease . in India, Ramalingam and his colleagues found infections with HTLV in a percentage 9.3% in the patients with severe hematological malignancies, and detected 3 of 22 (13.6%) patients with leukemia were infected with HTLV [10].
The author Dourado et al. mention that occurrences and infection with HTLV increased with age from 1 to 89 years old, going from 0.3 to 1.1 and then to 1.1 and 8.4% among those age group are 0–15, 16–30, 31–50 and > 50 years old, respect [11]
Also, the infection may increase in the population as such the study of Dourado et. al who found that occurrences of Human T lymphocyte virus type 1 was increased in females in low education state.

4. Conclusions

From our above results reaction showed that leukemia patients infected with HTLV in a prevalence 1.53% the prevalence of infected patients' chronic myeloid leukemia (CML) with HTLV were much more the prevalence of AML.

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