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Human *CCL3L1* gene expression in blood donors infected with HIV-1

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***Abstract*— The *CCL3L1* gene is very important in regulating of immunity against of pathogens. *CCL3L1* inhibits HIV-1 replication by competing with CCR5 co-receptors, which are essential receptors for virus entry into target cells. The aim of the study, which was done in Thi-Qar province from February 2022 to February 2023, was to monitoring the immune status by estimate the gene expression of the *CCL3L1* gene in people infected with HIV-1 compared to the group of control. Where 30 samples were collected from infected people and 30 samples from non-infected people as a control group. There was an increased level of gene expression in patients, where it was (17.02778) compared to the control group, which was (1.428108) using qPCR. There is a need for more molecular studies and follow-up of genetic and immunological changes in infected patients.**

***Keywords*— *CCL3L1*, *β- actin* , gene expression, HIV-1.**

I. INTRODUCTION

Chemokine ligand 3 like-1 (*CCL3L1*) codes the CCL3L1 protein [1], located on human chromosome 17 [2], which is the most powerful efficacious chemokine for HIV-1 suppression [1], it is plays an important role in regulatory of immune and host protective by production macrophage inflammatory proteins (MIP)-1α which has a role in decrease the expression of CCR5 co-receptor for entry HIV into cells [3]. There is association between *CCL3L1* expression and altered exposure to HIV-1. *CCL3L1* is critically important in HIV-1 pathogenesis. More chemokines mean less HIV-1 replication, suggesting that variances in the gene copies of immune response underlie different responses to infectious diseases. The *CCL3L1* expression levels are correlated with the number of copies for *CCL3L1* gene [4].The presence of a segment redundancy hotspot causes *CCL3L1* copy number to fluctuate widely. The chemokine co-receptor (CCR5) is naturally linked to *CCL3L1*. In addition to influencing HIV-1 susceptibility and possibly blocking HIV-1 entry by conquering required co-receptors, copy number variation (CNV) of *CCL3L1* is also closely related with HIV-1 exposure. When *CCL3L1* was low copy number that enhanced infection with HIV-1, as well as rapid progression to AIDS and death. CCR5 co-receptor ligand *CCL3L1* has genetic variations that affect its expression and function [2]. The *CCL3L1* variant range of *CCL3L1* transcripts has been documented from 0 to 14 transcripts in many different populations. *CCL3L1* copy numbers varied from 0 to 8 for the Malaysian and Indian populations and from 0 to 10 for the Chinese [3], where both the HIV-positive Malays and Indians had CN status at two, while the CN status of the Chinese had four [1], whereas most Europeans have between 0 to 5 copies [5].

African people have significant greater copies of *CCL3L1* compared to people from other continents populations, suggesting this gene confers resistance to HIV infection in these people [4]. HIV favorably selects people with a low concentration of the *CCL3L1* gene [6]. Given that individuals have fewer copies *CCL3L1* in population have an increase risk of infection with HIV as well as predisposition to the rapid development of AIDS, individuals with lower two of copies were more likely to be infected with HIV. For over 15 years of infection, however, the copy number of *CCL3L1* did not play a role in identifying infections. Additionally, the variation of *CCL3L1* have little influence on HIV-1 loading level, this difference in the *CCL3L1* version number does not appear to be the case the reason that regulates the diagnosis of chronic infection with HIV although it is related with exposure to HIV infection [2].

Check *CCL3L1* version in adolescents with early HIV infection Chronic stage without AIDS is no variance in CNV of *CCL3L1* distribution among infection with HIV-1 and control subjects it has also been reported that children with upper copy counts have a lower risk of transmitting HIV-1 from mother of infection [7].

CNV of *CCL3L1* rate in the Indian population ranges from 1- 6, risk of infected with HIV-1 was found to be associated with people who have less than two copies compared to those who have two or more copies [8]. The aim of current study to monitoring the immune status by estimate the gene expression of the *CCL3L1* gene in people infected with HIV compared to group of control.

II. MATERIALS AND METHODS

A. Samples

One ml of whole blood samples were collected from 30 individuals infected with HIV-1 and 30 individuals non-infected as control group, these samples were placed in EDTA tubes containing 1 ml of *TransZol*, in the main

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donation center in Thi-Qar province, from February 2022 to February 2023.

B. Methods

All samples were screening by using ELISA assay. Positive samples were collected from infected patients after their consent. Human RNA was isolated from 1ml of whole blood samples using the (TransGen biotech, Cat.No:ET101,China). Due to the company's guidelines. RNA converted to cDNA by using DiaStar™ RT Kit, SolGent, Cat.No.DR23-R10k, South Korea. Due to the company's guidelines. Nucleic acid amplification protocol qPCR is as follows: 95 °C at 10 min. for 1 cycle, 95 at 15 sec. 56 at 30 sec. 72 at 35 sec. for 45 cycle. Primer sequence of *CCL3L1* gene forward 5`-TCTCCACAGCTTCCTAACCAAGA -3`and reverse 5`-CTGGACCCACTCCTCACTGG -3` [9], Primer sequence of *β- actin* gene forward 5`- GAGCGCGGCTACAGCTT -3`and reverse 5`- TCCTTAATGTCACGCACGATTT -3` [10], with SYBR green dye.

Then note the gene expression through the difference between the control group and the patients after entering the Ct values into Excel 2010 by the equations:

Delta Ct = interested gene Ct – housekeeping gene Ct
Delta-delta Ct = delta Ct – average delta Ct of control
Fold change $= 2^{-\Delta\Delta Ct}$

III. RESULTS

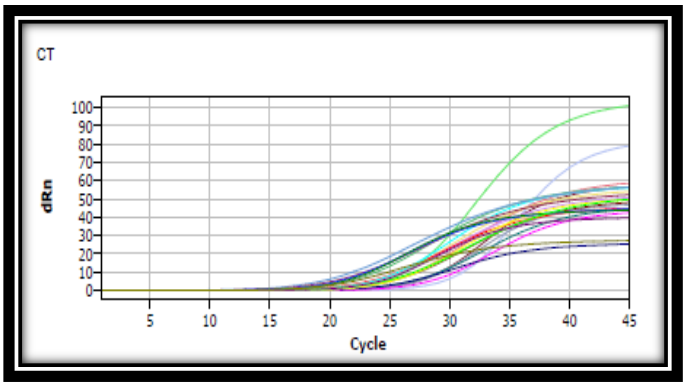
The results of gene expression showed a clear discrepancy between the expression of the *CCL3L1* gene in people infected with HIV-1 figure (1) compared to the control group figure (2), where the values of fold change for the infected were 17.03 while for the control group it was 1.43 as shown in the table (1) for control group samples and the table (2) for patient group samples:

TABLE (1) : Ct value for *CCL3L1* and *β-actin* genes, Delta ct , Delta ct average, Delta delta CT, fold change and average for control group:

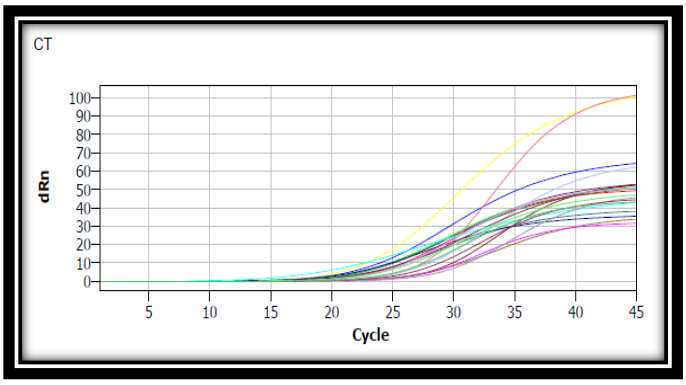
Co.	*CCL3L1*	*β-actin*	Dct	ddCT	F.change
1	24.98	19.83	5.15	3.944	0.064974
2	24.93	22.13	2.8	1.594	0.331252
3	22.23	20.56	1.67	0.464	0.724973
4	27.35	22.79	4.56	3.354	0.097801
5	17.9	17.1	0.8	-0.406	1.325007
6	28.11	21.54	6.57	5.364	0.024281
7	23.12	21.38	1.74	0.534	0.690637
8	25.8	24.06	1.74	0.534	0.690637
9	23.21	22.34	0.87	-0.336	1.262252
10	24.58	21.15	3.43	2.224	0.214047
11	23.18	23.04	0.14	-1.066	2.093621
12	21.29	21.16	0.13	-1.076	2.108183
13	20.29	20.26	0.03	-1.176	2.259494
14	20.31	20.12	0.19	-1.016	2.022304
15	22.31	21.54	0.77	-0.436	1.352848
16	23.27	22.96	0.31	-0.896	1.860899
17	24.23	24.11	0.12	-1.086	2.122846
18	23.52	23.49	0.03	-1.176	2.259494
19	20.82	20.74	0.08	-1.126	2.182528
20	20.02	19.54	0.48	-0.726	1.654047
21	19.22	18.43	0.79	-0.416	1.334223
22	23.72	23.67	0.05	-1.156	2.228387
23	24.62	24.59	0.03	-1.176	2.259494
24	21.13	20.43	0.7	-0.506	1.420107
25	19.36	19.29	0.07	-1.136	2.197708
26	20.44	20.42	0.02	-1.186	2.27521
27	23.14	22.22	0.92	-0.286	1.219255
28	20.42	19.38	1.04	-0.166	1.121943
29	19.02	18.15	0.87	-0.336	1.262252
30	25.23	25.15	0.08	-1.126	2.182528
total			36.18		42.84324
Dct average		1.206	average		1.428108

TABLE (2) : Ct value for *CCL3L1* and *β-actin* genes, Delta ct , Delta ct average, Delta delta CT, fold change and average for patient group:

Pa.	*CCL3L1*	*β-actin*	Dct	ddCT	F.change
1	23.73	28.8	-5.07	-6.276	77.49332
2	22.85	24.05	-1.2	-2.406	5.300028
3	20.62	26.59	-5.97	-7.176	144.6076
4	18.33	22.87	-4.54	-5.746	53.66836
5	19.34	22.63	-3.29	-4.496	22.56477
6	24.26	26.69	-2.43	-3.636	12.43212
7	19.98	24.13	-4.15	-5.356	40.95592
8	20.38	22.41	-2.03	-3.236	9.421782
9	23.59	26	-2.41	-3.616	12.26096
10	23.34	27.54	-4.2	-5.406	42.40022
11	21.01	25.85	-4.84	-6.046	66.07351
12	24.54	23.69	0.85	-0.356	1.279872
13	23.34	20.19	3.15	1.944	0.259895
14	25.65	23.98	1.67	0.464	0.724973
15	23.12	22.34	0.78	-0.426	1.343503
16	23.48	23.45	0.03	-1.176	2.259494
17	21.59	20.39	1.2	-0.006	1.004168
18	26.54	26.48	0.06	-1.146	2.212995
19	23.45	22.12	1.33	0.124	0.91764
20	21.39	19.73	1.66	0.454	0.730016
21	22.33	21.98	0.35	-0.856	1.810013
22	19.76	18.87	0.89	-0.316	1.244874
23	24.76	20.97	3.79	2.584	0.166778
24	21.98	20.54	1.44	0.234	0.850274
25	24.76	23.58	1.18	-0.026	1.018185
26	22.56	21.67	0.89	-0.316	1.244874
27	24.22	23.75	0.47	-0.736	1.665552
28	21.68	21.51	0.17	-1.036	2.050534
29	23.19	22.67	0.52	-0.686	1.608817
30	22.74	21.87	0.87	-0.336	1.262252
total					510.8333
average					17.02778



Figure(1):Ct value RT-PCR for *CCL3L1* gene in HIV patient.



Figure(2): Ct value RT-PCR for *CCL3L1* gene in control group.

IV. DISCUSSION

Since gene expression was further directly related to molecular function, studying gene expression variation within or among social people can yield more information about how human genes have evolved to perform their functions [11]. Gene expression levels do not differ significantly between one group of the population and another, with the exception of a few genes that show varying levels of expression between a particular population [12].

Molecular analyzes revealed that genes with high variability in expression within the population are associated with some human diseases, and among these diseases is HIV-1 infection, especially the first phase enter of virus into host cell [13]. This confirms what Kaslow *et al.*,[14], mentioned that there are certain genetic factors affecting exposure to infection with HIV-1 or the progress of AIDS, The clinical progression of HIV-1 infection was also noted to vary greatly between individuals. While some people with HIV develop AIDS very quickly, others are able to bring their virus under control and maintain a relatively stable immune system. Certain genes have been implicated in susceptibility to HIV/AIDS through genetic association analyses of AIDS patients.

Those genes control how HIV-1 enters cells and how the response to the virus, and significantly influence the susceptibility of individuals and populations to HIV-1[15].

One such gene is *CCL3L1*, which is highly variable due to the presence hotspot of segmental duplication [2]. It is a host factor that interacts with many HIV-1 genes, controls enter of virus and inhibits duplication, and shows high variability in expression between individuals [13]. *CCL3L1* prevents the virus from entering cells by competing with HIV-1 for the CCR5 receptor [16].

Through the current study regarding the gene expression of the *CCL3L1* gene between the infected group and the control group, it was found that there are clear expression levels of a gene among the infected compared to the control group using qPCR technique, this is consistent with Pilotti *et al.*, [9] who mentioned increase expression of the *CCL3L1* gene due to HIV-1.It also agrees with [13], described a 3-fold increase in the expression of *CCL3L1* gene.

However, the result of the current study differedform other studies that reported that there was no variance in *CCL3L1* levels among people HIV-1 patients and the control group [17].

Perhaps the reasons for the discrepancy in the results of these studies are due to the method of collecting or preparing samples, the use of inappropriate amounts of cDNA , or the inappropriate rounding to the nearest value as an integer [18]. Or the difference in the method of examination, as Urban *et al.*, [17], mentioned and there is a significant correlation between *CCL3L1* levels among people living with HIV-1 compared to the paralogue ratio test (PRT) control group, but there is no significant correlation when using the qPCR technique. However, qPCR is sometimes useful in studying gene expression [19,20].

Or, samples of studies that indicated there is no association between the *CCL3L1* and the HIV-1 may have been taken in the late stages of infection, as there is no effect of the *CCL3L1* on the HIV-1 in infected people for over 15 years [2].

V. CONCLUSION

It was found in current study there was an increase in *CCL3L1* gene expression in patients compared to the control group using qPCR. There is a need for more molecular studies and follow-up of genetic and immunological changes in infected patients.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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