WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 6, 112-121. Research Article ISSN 2277– 7105

LEVELS OF INTERLEUKIN-17 AND VITAMIN D AND THEIR CORRELATION IN VIRAL HEPATITIS PATIENTS

Ammar Hamza Abbas*[1], Dr. Mayada Noori Iqbal[1] and Dr. Leith Abdul Hussein Abdullah[2]

[1]College of Health and Medical Technology/Baghdad, Iraq.

[2]Gastroenterologist, Baghdad Teaching Hospital/Medical Complex, Baghdad, Iraq.

Article Received on
31 March 2017,

Revised on 21 April 2017,
Accepted on 11 May 2017

DOI:10.20959/wjpr20176-8554

***Corresponding Author**
Dr. Ammar Hamza Abbas
College of Health and Medical Technology/
Baghdad, Iraq.

ABSTRACT

Background: The interleukin 17 is usually linked to tissue damage in autoimmune disorders and bacterial and fungal infections. In both chronic Hepatitis B and Hepatitis C Virus infections, some reports show a close relationship between activation of Th17 lymphocytes and the extent of hepatic damage instigated by the antiviral immune response. Vitamin D deficiency has been often described in persons with chronic liver diseases. The strong Immunomodulatory effects of Calcitriol (active form of vitamin D) *in vitro*, suggested a possible therapeutic benefit in chronic hepatitis B infection and chronic hepatitis C cases. **Aims:** to investigate the sera levels of Interleukin-17 and Vitamin D3 (as Calcitriol) in viral hepatitis patients and determine the relationships between these biomarkers. **Methods:** Blood samples were taken from 70 chronic viral hepatitis patients and 35 healthy controls. The patients group consisted of 34 chronic HBV cases and 36 chronic HCV cases. All samples were quantified for (interleukin-17 and calcitriol) by sandwich Enzyme-linked immunosorbent assay. Non-parametric statistical tests (Kolmogorov-Smirnov test and Mann–Whitney test) were used by employing SPSS statistical software. **Results:** Results revealed that the mean of interleukin-17 sera of patients was (64.74ng/L) while it was (90.9ng/L) in controls with significant difference at ($P<0.05$). Furthermore, there was a highly significant difference ($P<0.01$) between the HBV and HCV groups, with means of (36.9ng/L and 70.1 ng/L) respectively. The mean of patients' serum calcitriol was significantly lower ($P<0.01$) than the controls' (79.05 and 113.3 pmol/L, respectively). The mean of calcitriol levels in HBV group was (44.88pmol/L), which is significantly lower ($P<0.01$) than the mean of HCV group (92.2pmol/L). There was a highly

significant positive relationship between 1,25 (OH)2D3 (Calcitriol) and interleukin-17 at p-value ($p< 0.01$). **Conclusion:** serum levels of interleukin-17 were low in patients but were higher in HCV patients than in HBV patients. Calcitriol and interleukin-17 are strongly correlated in a positive manner, which implies that Vitamin D3 had a suppressive effect on interleukin-17.

KEYWORDS: interleukin-17, HBV, HCV, Vitamin D3, calcitriol, viral hepatitis**.**

INTRODUCTION

Both Chronic hepatitis C virus (HCV) and hepatitis B (HBV) infections are responsible for about 57% of incidences of liver cirrhosis and 78% of primary liver cancer incidences globally and cause about a million mortalities per year.[1] Liver damage and disease progression are believed to be induced by host immune responses in both, HBV and HCV infections.[2]

The interleukin (IL-17) is a proinflammatory cytokine produced by $CD4^+$ T helper 17 cells via TCR activation and can stimulate the secretion of several other cytokines and chemokines from a variety of cells. IL-17 is usually linked to tissue damage in autoimmune disorders and bacterial and fungal infections [3]. IL-17 may also act as an important regulator for Th1 and cytotoxic T-lymphocyte (CTL) responses in inflammatory bowel disease (IBD) [4], anti-tumor immune responses[5], and intracellular bacterial infections.[6]

Vitamin D goes through significant biotransformation within the liver. Because of that, anomalous vitamin D metabolism can be assumed to be associated with chronic liver disease. Vitamin D deficiency has been often described in persons with chronic liver diseases.[7]

The existing evidence on the beneficial and harmful effects of vitamin D supplementation in individuals with chronic liver diseases is not sufficient and contrasting.[8] One meta-analysis research of observational and interventional studies in persons with HCV infection reported a positive relation between sustained virological response and high levels vitamin D.[9] On the other hand, one study reported association between vitamin D levels and chronic liver disease progression.[10]

The study aimed to investigate the levels of serum Interleukin-17 and Vitamin D3 (as Calcitriol) in serum of viral hepatitis patients and to assess the relationships between these biomarkers.

MATERIALS AND METHODS

Subjects

Seventy patients who were already diagnosed with chronic viral hepatitis B or C infection with ages (18-80) years were taken in this study. Thirty-five apparently healthy subjects with ages (3-80) years were also enrolled in the study as a control group. All patients were attending the advisory clinic of Baghdad teaching hospital and were previously diagnosed with chronic hepatitis.

Inclusion criteria were based on a positive enzyme-linked immunosorbent assay (ELISA) test and a positive PCR viral load test for HCV or HBV. An establishment of chronicity of the liver disease was done by abdominal ultrasounds, biochemical tests and clinical examination by the physicians at the clinic. Exclusion criteria were based on presence of other viral infections, history of smoking, recent disease onset (< 6 months) and other chronic diseases.

Measurement of interleukin-17 and calcitriol

All samples were quantified for (interleukin-17 and calcitriol) by using sandwich Enzyme-linked immunosorbent assay kits manufactured by Shanghai labs - China.

Statistical analysis

Non-parametric statistical tests (Kolmogorov-Smirnov test and Mann – Whitney test) were used by employing SPSS statistical software.

RESULTS AND DISCUSSION

Table (1) shows the baseline characteristics of the studied samples according to age, gender, and type of virus.

Table: 1 The baseline characteristics of the studied groups.

Variables		Patients			Controls
		HBV	HCV	Total	
Age (years)	Range	(18-70)	(23-80)	(18-80)	(3-80)
	Mean	37.83	45.96	37.89	42.06
Gender	Male No.(%)	18 (54%)	21(58%)	39(56%)	18(51.4%)
	Female No.(%)	16(46%)	15(42%)	31(44%)	17(48.6%)
Total No.		34	36	70	35

(*) NS: Non Sig. at P>0.05.

The study cinsist of a total of 105 individuals were taken in two groups, 70 patients with viral hepatitis B and C, and 35 apparently healthy controls. The patients group had 39 males (56%)

and 31 females (44%) while the control group had 18 males (51.4%) and 17 females (48.6%). 34 of the patients were infected with HBV while 36 patients had HCV infection. The mean age of the patients group was 37.89 years (37.83 for HBV and 45.96 for HCV) while the control group with mean age of 42 years. It is shown that the highest percentage of the patients group was males.

Levels of Interleukin 17 (ng/L) in studied groups

Descriptive statistics of serum levels of IL 17 (ng/L) are listed in table (2) which shows the summary of IL-17 levels in both patients and controls.

Table: 2 Sera levels of Interleukin 17 (ng/L) in studied groups.

Parameters	**Interleukin 17 (ng/L)**	
Groups	Patient	Control
Range	(17.36-785.87)	(32.28-664)
5% Trimmed Mean	64.744	90.996
Median	50.751	60.211
Interquartile Range	46.576	39.411
Mann-Whitney U test Asymp. Sig. (2-tailed)	Z = -2.139 P = 0.032 (S) (*)	

(*) **S: Sig. at P<0.05**

The mean of IL-17 in sera of patients was (64.74 ng/L) while it was (90.9 ng/L) in controls. The medians of the two groups were (50.75 and 60.21) ng/L respectively. Moreover, the p-value of the difference between the patients and controls was (0.032) which is significant.

Table 3 shows the serum levels of IL-17 within the patients group and the comparison between the HBV and HCV groups.

The mean of IL-17 serum levels in HBV group was (36.9ng/L), which is nearly half of the HCV group's mean (70.1ng/L). The medians had a similar behavior to the means, which were (28.59 and 60.58) ng/L respectively. The p-value of the difference between the two groups was highly significant (0.0).

Present results indicated that patient group had a lower trimmed mean than the control group, this may be attributed to their responsiveness to treatment or efficiency in the immune system because treatment responders tend to have lower IL 17 expression as concluded by an Iranian study done in 2016,[11] That result suggests a shift in immunity towards the TH1 side rather than TH 17.

Table 3: sera levels of IL-17 (ng/L) in patients group.

Descriptive statistics	IL-17 (ng/L)	
	HBV group	HCV group
Range (Max.-Min.)	(150.66-17.36)	(785.87-45.53)
5% Trimmed Mean	36.99	70.11
Median	28.59	60.58
Interquartile Range	21.34	31.33
Mann-Whitney U test Asymp. Sig. (2-tailed)	Z = - 4.321 P = 0.000 (Highly significant)	

It was also suggested that antiviral treatment lowers the levels of IL-17 in patients' serum.[12],[13] This decrease in IL-17 seems to favor the survival of subjects under treatment.[14]

The differences between the means of the patients and control groups (Z = -2.139) were significant at ($p<0.05$) which is most likely due to the important role that IL 17 plays during the chronic viral infection in indicating liver damage as explained previously in the literature review chapter.[12]

Results also showed a highly significant decrease ($P <0.01$) in trimmed mean of IL-17 serum levels in HBV patients when compared to HCV patients group (Z = - 4.321). In agreement, Sousa[15] found the mean of IL-17 serum level in HCV patients to be (96.8ng/L) while Du[16] found the mean of Il-17 in HBV patients to be (38.9 ng/L), which is lower than in HCV cases. The reason of this difference could be attributed to stimulation of Th17 cells by the HCV through expression of thymic stromal lymphopoietin (TSLP) which is essential factor in the differentiation and proliferation of Th17 cells.[13]

Levels of Calcitriol (Vitamin D3) (pmol/L) in studied groups

Descriptive statistics of the Calcitriol (pmol/L) parameter are listed in table (4) which shows the summary statistics of the studied parameter in patient and control groups.

Table: 4 Sera levels of Calcitriol (pmol/L) in the studied groups.

Parameters	Calcitriol (pmol/L)	
Groups	Patient	Control
Range	(20.1-631.31)	(39.14-546.67)
5% Trimmed Mean	79.055	113.317
Median	62.82	87.74
Interquartile Range	73.945	43.679
Mann-Whitney U test Asymp. Sig. (2-tailed)	Z = -2.683 P = 0.007 (HS) (*)	

(*) HS: Highly Sig. at P<0.01

The table shows that the patients group had a trimmed mean of (79.055 pmol/L) while the controls had trimmed mean of (113.317 pmol/L) for serum Calcitriol. Additionally, the medians of the two groups were (62.82 and 87.74) pmol/L respectively. The p- value of differences between the two groups was highly significant (0.007).

Table 5 shows the levels of Calcitriol in HBV and HCV infected subjects within the patients group.

Table: 5 Sera levels of Calcitriol (pmol/L) in the patients group.

Descriptive statistics	Calcitriol (pmol/L)	
	HBV group	HCV group
Range	(20.1-229.64)	(37.1-543.8)
5% Trimmed Mean	44.88	92.2
Median	30.1	85.1
Interquartile Range	39.54	59.7
Mann-Whitney U test Asymp. Sig. (2-tailed)	Z = - 4.136 P = 0.000 (HS) (*)	

(*) HS: Highly Sig. at P<0.01

The table shows that the mean of HBV (44.88 pmol/L) is nearly half of the mean of HCV group (92.2pmol/L), with the medians of the two groups (30.1 and 85.1) pmol/L respectively. The Mann-Whitney p-value of the two groups was highly significant (0.000).

Present results indicated high differences between patients and controls when comparing the two means (Z= -2.683) thereby establishing that the two groups were significantly different (P = 0.007). They also showed decreased levels of Calcitriol in patients group when compared to controls which is in agreement with an American study in 2010[17] that found high prevalence of vitamin D deficiency in CLD patients in comparison to controls. This was most likely due to decreased liver function in chronic liver disease patients and possibly some degree of liver fibrosis.[18]

Results also indicated a significant difference in the serum levels between the two groups with a higher trimmed mean in HCV group but both are below the mean of the control group. Similar to this result, the reported mean of vitamin D by[19] of HBV patients were lower than the reported mean of vitamin D by[20] of HCV patients, both in Egypt.

This difference is due to vitamin D3 being more related to the viral load of HBV rather than the HCV viral load, indicating a functional relationship between it and HBV load.[21],[22] Also reported that vitamin D levels were not related to HCV load in patients (p= 0.903) which consolidates the current result.

Pearson's Correlation Coefficients between studied parameters

Table (6) shows Pearson's correlation coefficients, with their significance level among the studied parameters (IL-17 and Calcitriol), in patients, and control groups.

Table: 6 Pearson's Correlation Coefficients between studied parameters in each group.

Groups	Studied Parameters		IL-17
Controls	Calcitriol (pmol/L)	Pearson Correlation	0.229
		Sig. (2-tailed)	0.186
Patients	Calcitriol (pmol/L)	Pearson Correlation	0.970
		Sig. (2-tailed)	0.000*

(*) Correlation is significant at the P< 0.01 level (2-tailed).

Results show that weak correlations were accounted with no significance at P>0.05 between studied parameters in control group, while within patients group, strong, highly significant and positive correlations were accounted between Calcitriol (1,25 dihydroxy Vit. D3) and IL-17 ($p<0.01$).

As we have previously known in the literature review chapter that 1,25-dihyroxyvitamin D3 or Calcitriol is the active metabolite of Vitamin D.[23] And some patients may have low Vitamin D level while having normal or even elevated serum levels of 1,25 dihyroxyvitamin D3. Therefore, it does not reflect the body stores of Vitamin D but is used to examine the activation level of Vitamin D.[24]

Present results showed strong and highly significant positive correlations between levels of Calcitriol and IL-17 ($p<0.01$). This is most likely due increased activation of Vitamin D into 1,25(OH)2D3 (Calcitriol) by activated T-cells, Dendritic Cells (and probably also B cells).[25] This increased activation is most probably to counteract the increased expression levels of IL-17 because Calcitriol was found to suppress inflammatory cytokines such as IL-17 while selectively promoting anti-inflammatory cytokines like IL-10 and promoting expression of CTLA-4 and FoxP3 in-Vitro.[26]

Another study in Egypt agreed with this result and found that Vitamin D suppresses IL-17 in Patients with Chronic Hepatitis C Liver Disease.[19] Also, a research found that the anti-inflammatory effect of Calcitriol caused an improvement in the therapeutic response to Interferon and Ribavirin treatment in chronic hepatitis Patients.[27]

CONCLUSION

Serum IL-17 was lower in patients group relative to controls, but was higher in HCV patients than in HBV patients. Calcitriol was lower in patients group; this decrease was more evident in HBV group than in HCV-infected group, which suggests a functional relationship between HBV and Calcitriol serum levels. Patients with increased serum IL-17 had high activation levels of vitamin D evidenced by increased serum 1,25 (OH)2D3 (Calcitriol) which implies that Vitamin D3 had a suppressive effect on IL -17.

REFERENCES

1. Rehermann B. Pathogenesis of Chronic Viral Hepatitis: Differential Roles of T cells and NK cells. *Nature medicine,* 2013; 19(7): 859-868. doi:10.1038/nm.3251.
2. Rehermann B, Hepatitis C. virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. J Clin Invest, 2009; 119: 1745–1754.
3. Dong C. Regulation and pro-inflammatory function of interleukin-17 family cytokines. *Immunological Reviews,* 2008; 226(1): 80–86.
4. Feng, T., Qin, H., Wang, L., Benveniste, E.N., Elson, C.O. and Cong, Y., Th17 cells induce colitis and promote Th1 cell responses through IL-17 induction of innate IL-12 and IL-23 production. *The Journal of* Immunology, 2011; 186(11): 6313-6318.
5. Ma, Y., Aymeric, L., Locher, C., Mattarollo, S.R., Delahaye, N.F., Pereira, P., Boucontet, L., Apetoh, L., Ghiringhelli, F., Casares, N. and Lasarte, J.J., Contribution of IL-17–producing γδ T cells to the efficacy of anticancer chemotherapy. *Journal of Experimental Medicine,* 2011; jem-20100269.
6. Lin, Y., Ritchea, S., Logar, A., Slight, S., Messmer, M., Rangel-Moreno, J., Guglani, L., Alcorn, J.F., Strawbridge, H., Park, S.M. and Onishi, R., Interleukin-17 is required for T helper 1 cell immunity and host resistance to the intracellular pathogen Francisella tularensis. *Immunity*, 2009; *31*(5): 799-810.
7. Skaaby, T., Husemoen, L.L.N., Borglykke, A., Jørgensen, T., Thuesen, B.H., Pisinger, C., Schmidt, L.E. and Linneberg, A., Vitamin D status, liver enzymes and incident liver disease and mortality: a general population study. *Endocrine*, 2014; *47*(1): 213-220.

8. Bjelakovic, G., Nikolova, D., Bjelakovic, M. and Gluud, C., Vitamin D supplementation for chronic liver diseases in adults. *The Cochrane Library*, 2015.
9. Villar, L.M., Del Campo, J.A., Ranchal, I., Lampe, E. and Gomez, M.R., Association between vitamin D and hepatitis C virus infection: a meta-analysis, 2013.
10. Corey, K.E., Zheng, H., Mendez-Navarro, J., Delgado-Borrego, A., Dienstag, J.L., Chung, R.T. and HALT-C Trial Group, Serum vitamin D levels are not predictive of the progression of chronic liver disease in hepatitis C patients with advanced fibrosis. *PLoS One*, 2012; *7*(2): 27144.
11. Borzooy, Z., Streinu-Cercel, A., Mirshafiey, A., Khamseh, A., Mahmoudie, M.K., Navabi, S.S., Nosrati, M., Najafi, Z., Hosseini, M. and Jazayeri, S.M., IL-17 and IL-22 genetic polymorphisms in HBV vaccine non-and low-responders among healthcare workers. *Germs*, 2016; *6*(1): 14.
12. Meng, P., Zhao, S., Niu, X., Fu, N., Su, S., Wang, R., Zhang, Y., Qiao, L. and Nan, Y., Involvement of the interleukin-23/interleukin-17 axis in chronic hepatitis C virus infection and its treatment responses. *International Journal of Molecular Sciences*, 2016; *17*(7): 1070.
13. Lee, H.C., Sung, S.S.J., Krueger, P.D., Jo, Y.A., Rosen, H.R., Ziegler, S.F. and Hahn, Y.S., Hepatitis C virus promotes t-helper (Th) 17 responses through thymic stromal lymphopoietin production by infected hepatocytes. *Hepatology*, 2013; *57*(4): 1314-1324.
14. Yu, X., Guo, R., Ming, D., Su, M., Lin, C., Deng, Y., Lin, Z. and Su, Z., Ratios of regulatory T cells/T-helper 17 cells and transforming growth factor-β1/interleukin-17 to be associated with the development of hepatitis B virus-associated liver cirrhosis. *Journal of gastroenterology and hepatology*, 2014; *29*(5): 1065-1072.
15. Sousa, G.M., Oliveira, I.S., Andrade, L.J., Sousa-Atta, M.L.B., Paraná, R. and Atta, A.M., Serum levels of Th17 associated cytokines in chronic hepatitis C virus infection. *Cytokine*, 2012; *60*(1): 138-142.
16. Du, W.J., Zhen, J.H., Zeng, Z.Q., Zheng, Z.M., Xu, Y., Qin, L.Y. and Chen, S.J., Expression of interleukin-17 associated with disease progression and liver fibrosis with hepatitis B virus infection: IL-17 in HBV infection. *Diagnostic pathology*, 2013; *8*(1): 40.
17. Arteh J, Narra S, Nair S. Prevalence of vitamin D deficiency in chronic liver disease. Dig Dis Sci., 2010; 55: 2624–2628.
18. Terrier, B., Carrat, F., Geri, G., Pol, S., Piroth, L., Halfon, P., Poynard, T., Souberbielle, J.C. and Cacoub, P., Low 25-OH vitamin D serum levels correlate with severe fibrosis in

HIV-HCV co-infected patients with chronic hepatitis. *Journal of hepatology*, 2011; *55*(4): 756-761.

19. Reda, R., Abbas, A.A., Mohammed, M., El Fedawy, S.F., Ghareeb, H., El Kabarity, R.H., Abo-Shady, R.A. and Zakaria, D., 2015. The interplay between zinc, vitamin D and, IL-17 in patients with chronic Hepatitis C liver disease. *Journal of immunology research*, *2015*.
20. Eltayeb, A. A., Abdou, M. A. A., Abdel-aal, A. M., & Othman, M. H. Vitamin D status and viral response to therapy in hepatitis C infected children. *World Journal of Gastroenterology : WJG*, 2015; *21*(4).
21. Farnik, H., Bojunga, J., Berger, A., Allwinn, R., Waidmann, O., Kronenberger, B., Keppler, O.T., Zeuzem, S., Sarrazin, C. and Lange, C.M., Low vitamin D serum concentration is associated with high levels of hepatitis B virus replication in chronically infected patients. *Hepatology*, 2013; *58*(4): 1270-1276.
22. Yu, R., Sun, J., Zheng, Z., Chen, J., Fan, R., Liang, X., Zhu, Y., Liu, Y., Shen, S. and Hou, J., Association between vitamin D level and viral load or fibrosis stage in chronic hepatitis B patients from Southern China. *Journal of gastroenterology and hepatology*, 2015; *30*(3): 566-574.
23. Holick MF. Vitamin D deficiency. N. Engl. J. Med., 2007; 357: 266–281.
24. Kennel, Kurt A., Matthew T. Drake, and Daniel L. Hurley. "Vitamin D Deficiency in Adults: When to Test and How to Treat." *Mayo Clinic Proceedings*, 2010; 85.8: 752–758.
25. Sigmundsdottir, H., Pan, J., Debes, G.F., Alt, C., Habtezion, A., Soler, D. and Butcher, E.C., DCs metabolize sunlight-induced vitamin D3 to'program'T cell attraction to the epidermal chemokine CCL27. *Nature immunology*, 2007; *8*(3): 285-293.
26. Jeffery, L.E., Burke, F., Mura, M., Zheng, Y., Qureshi, O.S., Hewison, M., Walker, L.S., Lammas, D.A., Raza, K. and Sansom, D.M., 1, 25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. *The Journal of Immunology*, 2009; *183*(9): 5458-5467.
27. Iruzubieta, P., Terán, Á., Crespo, J. and Fábrega, E., Vitamin D deficiency in chronic liver disease. *World journal of hepatology*, 2014; *6*(12): 901.