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MOLECULAR STUDY OF VDR POLYMORPHISMS AND THEIR GENOTYPING ASSOCIATION AS PREDICTOR RISK FOR HEPATITIS B AND C INFECTION

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ABSTRACT : Biological and epidemiological data suggest that vitamin D levels may influence cancer development. Several single nucleotide polymorphisms have been described in the vitamin D receptor (VDR) gene in association with cancer risk. Vitamin D exerts immunomodulatory effects on the host response against infection with hepatitis virus. Numerous studies focus on relationship between vitamin D receptor (VDR) polymorphisms (*FokI, BsmI*) and the risk of HBV infection in different ethnic groups. The aim of this study was to evaluate the possible association between the vitamin D receptor (VDR), single-nucleotide polymorphisms (SNPs) in patients with hepatitis B and C virus (HBV and HCV) infection. Study subjects were divided into three groups: 94 HBV patients, 109 HCV, and 82 healthy controls. The VDR polymorphisms were genotyped using PCR-RFLP by *BsmI* enzyme. The Genotype frequency of polymorphisms of (VDR) gene in Hepatitis B, C and Control, it was revealed that CC allele was higher than others 58.54% in control, 54.26% in HBV and56.88% in HCV respectively. Results of Allele frequency showed that T allele was higher than C (79.27% in control, 75.53% HBV and 72.02% HCV). We conclude that the VDR polymorphisms may contribute to increased susceptibility to HBV and related HCV in the Babylon population. Due to the marginal significance, further large and well-designed studies in diverse ethnic populations are needed to confirm our results.

Key words : HCV, HBV, VDR, polymorphism.

INTRODUCTION

As cited in various research articles that HCV has caused massive impact onpublic health and around 170 million people in the world are infected with HCV, with an estimated 3 to 4 million new infections global per year (Kamal, 2008). It is affects more than 4.6 million people in the United States (Edlin *et al*, 2015) and is associated with more than 15,000 deaths annually (Ly kn, 2012 and Al-Marzoqi, 2015).

Vitamin D is involved in the metabolism of skeleton as a systemic hormone but also has important roles in the regulation of host immune responses and development of cancer (Haussler *et al*, 1998). For example, vitamin D inhibits lymphocyte proliferation, stimulates monocyte differentiation and exhibits antiproliferation activities in several types of cancer cells (Uitterlinden *et al*, 2004). The active form of vitamin D, 1, 25-dihydroxyvitamin D, exerts immunomodulatory effects by the vitamin D receptor (VDR) (Haussler *et al*, 1998) and high concentration of VDR is detected in the macrophages and T lymphocytes, especially CD8-positive lymphocytes. The VDR locus is located at chromosome 12q13.1 with a size of more than 100 kb. Three adjacent restriction polymorphic sites in the VDR gene, the BsmI (rs1544410, A–G base change, designate as genotype B/B, B/b, b/b), ApaI (rs7975232, G–T base change, designate as genotype A/A, A/a, a/a) and TaqI (rs731236, T–C base change, designate as genotype T/T, T/t, t/t) have been reported and extensively studied in several diseases. Although, VDR gene variant of genotype t/t was reported to be associated with HBV clearance and active form of vitamin D was shown to inhibit HCC cell proliferation *in vitro* and *in vivo* (Pourgholami *et al*, 2000).

Results for a lot of studies indicated that a decrease in vitamin D levels could reflect the severity of hepatocellular injury and so serve as a new hepatic biomarker in progressive liver diseases (El Husseiny *et al*, 2012). Even so, these findings are consistent with previous results, indicating that Vitamin D inadequacy is common in non-cholestatic chronic liver diseases and correlates with disease severity (Babbs *et al*, 1988; Fisher and Fisher, 2007). In other earlier studies, a deficient vitamin D status was linked to severe fibrosis and low sustained virologic responses (SVR) during interferon

(IFN)-γ-based therapies (Petta *et al*, 2010 and Israa, 2018).

MATERIALS AND METHODS

Study subjects

The practical side of this study was done during the period from October 2017 to March 2018. Two hundred and eighty five samples were collected. Two enrolled groups of subjects were involved in this study.

Patients

This study included 203 patients with hepatitis infection, 94 patients with hepatitis B virus infection and 109 patients with hepatitis C virus infection admitted to Margan hospital, Center of liver diseases and gastrointestinal system. Patients included (130 males and 73 females), with an age range (HBV: 44.6 ± 8.2), (HCV: 45.3±13.3) and (Control: 49.2±9.04) years, they were diagnosed by serological and molecular tests and selected in the current study. Blood and serum samples taken from every patient and control having thoroughly examined.

Healthy control group

Eighty two actual healthy persons from various Iraqi populations were arbitrarily involved in this study.

Blood sampling

About five milliliters of venous blood were collected from each patient in this study. The blood was divided into two parts: one part (about two milliliters) was collected into EDTA containing tubes for genetic part. The second part of the blood was placed in gel tube for thirty minutes, then transferred to plain tube and serum was obtained by centrifugation at 3000 rpm for 15 min; after that the serum collected and kept in the freezer (-20 ºC) until it was used for the immune and viral assay.

Isolation of genomic DNA

Genomic DNA was used for molecular study by sequestered from the fresh blood, which collected in tubes of anti-coagulant EDTA and for frozen blood samples we recommended using protease K were applied using for DNA purification; Favor prep Blood genomic DNA purification kit. The isolation of DNA depended on the 5 stage procedure utilizing salting out techniques (Sambrook and Manianatis, 1989 and Al-Marzoqi, 2018) :

- Lysis of the RBCs in the Cell Lysis Solution.
- Lysis of the WBCs and their nuclei in the Nuclei Lysis Solution.
- A salt out precipitation step using the Protein Precipitation Solution then removed the cellular proteins.
- The genomic DNA was concentrated and desalted by Isopropanol precipitation.
- The genomic DNA was rehydrated using the DNA Rehydration Solution.

Isolation kit components

Components	Amount	Components	Amount
Cell Lysis Solution	500 ml	Protein Precipitation Solution	125 ml
Nuclei Lysis Solution	250 ml	DNA Rehydration Solution	100 ml

The Protocol for DNA separation

Procedure, which favor prep kit recommend for DNA separation as reveled in protocol.

The Estimation of DNA concentration and purity

The DNA concentration of samples was estimated by using the Nano drop by putting 2.5µl of the extracted DNA in the machine to detect concentration in ng/µL and the purity detected by noticing the ratio of optical density (OD) 260/280 nm to detect the contamination of samples with protein. The accepted 260/280 ration for purifying DNA was between 1.7-1.9 (Sambrook and Russell, 2001; Ali, 2018).

Electrophoresis of agarose gel

Agarose gel electrophoresis was embraced to affirm the nearness and uprightness of the separated DNA after genomic DNA extraction (Sambrook and Maniatis, 1989).

Protocol of Gel electrophoresis

Borate EDTA Buffer preparation (1X TBE)

This solution was prepared by adding 900 ml Distill water to 100 ml 10X TBE (Promega, Germany), forming 1 liter of (1x) TBE buffer (Sambrook and Russel, 2001; Abeer, 2018).

Preparation of agarose gel

- The amount of 1 X TBE (100 ml) was taken in a beaker
- Agarose powder (1.5 gm) was added to the buffer
- The solution was heated to boiling using a microwave oven for 2 min.
- Ethidium Bromide (1 µl) of (10mg/ml) was added to the agarose solution.
- The agarose was stirred in order to be mix and avoid making bubbles.
- The solution was left to cool down at 50–60°C.

Casting of the horizontal agarose gel

Subsequent to settling the brush in 1 cm a long way from one edge, the agarose game plan was filled the gel

plate. The agarose was allowed to bond at room temperature for 30 minutes. The modified brush was absolutely removed and the gel plate was set in the gel tank. The tank was stacked with 1 X TBE support until it accomplished 1-2 mm over the surface of the gel.

DNA loading & electrophoresis

DNA (3 µl) was mixed with (2 µl) loading dye. The samples loaded carefully into the individual wells of the gel, and then electrical power was turned on at 70 volt for 1 hour, afterwards the DNA moved from cathode (-) to anode (+) poles. The Ethidium Bromide stained bands in the gel were visualized using UV. Trans illuminator at 350 nm and photographed (Ammar, 2018).

PCR technique

Genotype was determined using PCR amplification followed by restriction fragment length polymorphisms; RFLP assay (Kamen and Tangpricha, 2010). Three fragments of VDR gene were amplified; BsmI fragment (800 bp, 650 bp and 150). Sets of primer manufactured by Ligo, USA. The primer sequences 5'-CAACCAAGACTCAAGTACCGCGTCAGTGA-3' and 5'-AACCAGCGGAAGAGGTCAAGGG-3. PCR optimization was done as a first step by using a gradient temperature. This is highly important to determine the optimum annealing temperature. The PCR reaction mixture for gradient consisted of 5µl template DNA, 5µl master mix, 5µl of each forward and reverse primer in 20 µl of total reaction volume. PCR condition of gradient is shown in Table 1.

After resolving of optimum annealing temperature for VDR gene by selecting the clearest and, which is 63°C, PCR mixture was 5µl DNA, 5µl master mix, 1.5 forward and reverses primer. PCR condition for VDR was performed as in Table 1. Results of PCR amplicons were produce 800 bp. The *BsmI* restriction site resulted in two fragments (650 bp and 150 bp). The resulting 800 bp PCR product is then digested with *BsmI* at 65 C for 18 h using 5 units of enzyme (BIOLAB, UK) per 20 ll reactions. Following digestion, the DNA fragments were separated using 2% agarose gel containing ethidium bromide then, visualized under shortwave UV light and compared to those of DNA ladder run at the same time.

RESULTS AND DISCUSSION

In Table 2, which describes the genotype frequency of polymorphisms of (VDR) gene in Hepatitis B, C and Control, it was revealed that CC allele was higher than others 58.54% in control, 54.26% in HBV and 56.88% in HCV respectively. Results of Allele frequency showed that T allele was higher than C (79.27% in control, 75.53% HBV and 72.02% HCV).

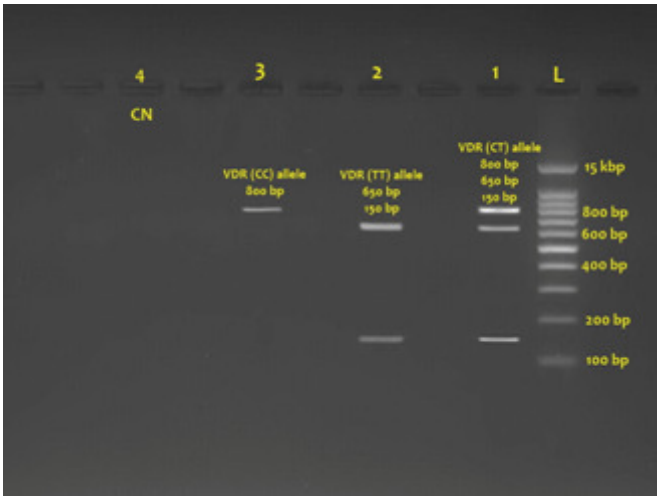

Fig. 1 : The Electrophoresis Pattern of VDR gene Polymorphisms. L lane contain the 100 bp DNA Ladder, 5% NuSieve® 3:1 agarose gel in 1X TBE buffer containing 0.5µl ethidium bromide.
(1) Lanes positive results for **CT** genotype with 800, 650 and 150 bp treated with BsmI enzyme.
(2) Lanes positive results for **TT** genotype with 650 and 150 bp treated with BsmI enzyme.
(3) Lanes positive results for **CC** genotype with 800 bp treated with BsmI enzyme.
(4) Lane was control negative of PCR product.

Vitamin D wasn't an independent factor that determined treatment response; this has also been reported for chronic HCV patients (Farid *et al*, 2016). Such results disagree with previous studies that reported serum vitamin 25(OH) D3 level was an independent factor that significantly contributed to sustained virological response (Arai *et al*, 2015). Vitamin D binds to VDRs on the surfaces of monocytes and lymphocytes, activating the innate immunity systems and enhancing immune responses by inhibiting Th1 cell functions and activating Th2 cell responses (Beard *et al*, 2011; Hussein, 2018 and Hewison, 2010).

The inhibitory role of vitamin D in viral replication is still unclear; it may directly inhibit viral replication through up-regulation of IFN-β expression (Gal Tanamy *et al*, 2011) or by inhibiting a viral assembly step (Matsumura *et al*, 2012). More studies are needed to explore its exact molecular effect on viral replication and infection susceptibility. Vitamin D is involved in the metabolism of skeleton as a systemic hormone but also has important roles in the regulation of host immune responses and development of cancer (Haussler *et al*, 1998). For example, vitamin D inhibits lymphocyte proliferation, stimulates monocyte differentiation and exhibits antiproliferation activities in several types of cancer cells (Uitterlinden *et al*, 2004 and Hassan, 2016).

The active form of vitamin D, 1, 25 dihydroxyvitamin D, exerts immunomodulatory effects by the vitamin D

receptor (VDR), (Haussler *et al*, 1998) and high concentration of VDR is detected in the macrophages and T lymphocytes, especially CD8-positive lymphocytes (Veldman *et al*, 2000). The VDR locus is located at chromosome 12q13.1 with a size of more than 100kb. Association studies of several polymorphisms in the VDR gene have been performed to investigate their implication with severity of chronic liver disease (Huang *et al*, 2010; Israa, 2016 and Yao *et al*, 2013).

One of the common genetic variations of VDR gene is the bat-haplotype consisting of BsmI, ApaI and TaqI (Uitterlinden *et al*, 2004). These genetic variations have been described as important modulators of several chronic liver diseases such as primary biliary cirrhosis and autoimmune hepatitis (Vogel *et al*, 2002 and Fan *et al*, 2005). Although, VDR gene variant of genotype t/t was reported to be associated with HBV clearance and active form of vitamin D was shown to inhibit HCC cell proliferation *in vitro* and *in vivo* (Bellamy *et al*, 1999; Israa Hussein, 2016 and Pourgholami *et al*, 2000).

The association of VDR gene polymorphisms with distinct clinical phenotypes of chronic HBV carriers remain largely unclear. Taking advantage of rampant HBV infection in Taiwan, the aim of this study is to investigate the association of VDR gene polymorphisms with distinct clinical phenotypes of Taiwanese chronic HBV carriers as well as the risk of Hepatitis development. Recent studies have reported the relationship between VDR gene polymorphisms and Hepatitis development in patients with chronic HCV infection (Falleti *et al*, 2010 and Lange *et al*, 2013).

Table 1 : PCR condition for VDR.

Step	Temperature (°C)	Time/min.	Cycles
Initial denaturation	94	4	1
Denaturation	94	45s	40
Annealing	63	40s	40
Extension	72	1	40
Final extension	72	7	1
Storage	4	∞	

Falleti *et al* (2010) have demonstrated that VDR genetic polymorphisms are significantly associated with the occurrence of HCC in cirrhotic patients, who underwent liver transplantation. The significant association between the polymorphisms in VDR, which serves as the physiological target to mediate vitamin D effects and HCV-induced HCC suggests that an impaired vitamin D metabolism contributes to hepatocarcinogenesis in chronic HCV infection. Although, serum vitamin D levels and history regarding vitamin D intake (dietary or supplemental) were not available, this could be justified since VDR gene variants modulate biological effects of vitamin D without influencing vitamin D plasma levels (Uitterlinden *et al*, 2004 and Wang *et al*, 2010). At the 32 -end of the VDR gene, a Bsm1 restriction fragment length polymorphism (RFLP) is found, which is strongly associated with other polymorphisms (Whitfield *et al*, 2001).

Early study showed that VDR t/t genotype was associated with protection from chronic HBV infection (Bellamy *et al*, 1999). This study confirmed that there was no t/t genotype in the chronic carriers of HBV (Suneetha *et al*, 2006). Other study also revealed the association of VDR gene polymorphisms with HBeAg positivity. HBeAg is a serological marker of active HBV replication, and seroconversion of HBeAg usually confers a favorable outcome in Asian HBV carriers (Chu, 2000 and Lena, 2017).

This finding implies the association of VDR gene with the replication activity of HBV, thus HBV carrier with the genotype or haplotype associated with HBeAg positivity, that is, genotype B/b, B/B, T/t or haplotype b/A, B/a, B/A, B/T, B/t, A/t, b/A/T, B/a/T, B/A/T, B/A/t and b/A/t, should also be closely followed up to decide the best time for treatment. As shown in the association of VDR gene polymorphisms with other diseases (Valdivielso and Fernandez, 2006).

The effect of VDR gene polymorphisms on the course of HBV infection may be different in different ethnicities.

Table 2 : Genotype frequency of polymorphisms of (VDR) gene in Hepatitis B, C and Control.

Genotype	Control (82)		Hepatitis B (94)		Hepatitis C (109)		P value
	No	%	No	%	No	%	
CC	48	58.54	51	54.26	62	56.88	0.363
TC	34	41.46	40	42.55	33	30.28	0.669
TT	0	0.00	3	3.19	14	12.84	0.0002
Total	82	100.00	94	100.00	109	100.00	
Allele frequency							
T	130	79.27	142	75.53	157	72.02	0.278
C	34	20.73	46	24.47	61	27.98	0.020
Total	164	100.00	188	100.00	218	100.00	

These include genotype B/b, B/B, T/t or haplotype b/A, B/a, B/A, B/T, B/t, A/t, b/A/T, B/a/T, B/A/T, B/A/t or b/A/t. Individuals with these genotype or haplotype are prone to HBV infection through favorable replication of the virus. Individual with genotype B/b, haplotype B/a, B/T and B/a/T are especially prone to HBV infection and replication due to less active immune response against the virus as reflected by the less hepatitis flare in these patients. In addition, although individuals with genotype T/t, haplotype A/t and b/A/t have frequent hepatitis flares, the immune response may be too weak to inhibit HBV replication that leads to persistent positivity of HBeAg No association of VDR BsmI, ApaI and TaqI polymorphism with the risk of HCC was found in this study, suggesting a minimal role of VDR gene polymorphisms in hepatocarcinogenesis. However, the anti-proliferative effects of active form of vitamin D against HCC cell had been reported to correlate with intracellular VDR level (Pourgholami and Morris, 2004). A vitamin D analog, Seocalcitol, had been used to treat patients with inoperable HCC (Dalhoff *et al*, 2003). Nevertheless, no further controlled studies have been carried out. Further studies are needed to clarify the role of vitamin D and VDR in the development and control of HCC. The predication of the HBV response to PEG-IFN may allow clinicians to minimize HBV infection complications and utilize effective selection antiviral drugs at the initiation of treatment, minimizing drug related side effects and decreasing overall costar the most commonly genotyped SNPs of VDR (Vu *et al*, 2013).

In our study, patients with the CC allele in the VDR BSMI SNP responded more to PEG-IFN treatment than those with CT and TT alleles as evident (Abdelsalam *et al*, 2016). Similarly, Li *et al* (2014) that found in their study the CC allele in the VDR responded more to PEG-IFN treatment than those with CT and TT alleles as evident. Vitamin D levels exhibited high sustained virological responses (better treatment response) among HCV individuals (Villar *et al*, 2013).

Although, the vitamin D levels were lower in HBV patients than in healthy controls In the present study, although the vitamin D levels were lower in HBV patients than in healthy controls, vitamin D wasn't an independent factor that determined treatment response; this has also been reported for chronic HCV patients (Farid *et al*, 2016). Such results disagree with previous studies that reported serum vitamin 25(OH) D3 level was an independent factor that significantly contributed to sustained virological response (Arai *et al*, 2015). The primers were supplied by Ligo (USA) Organization as a lyophilized result of various picomols fixations. Lyophilized preliminary was disintegrated in a free DNase/RNase water to give a final concentration of 100 pmol/µl and kept as a stock in -20ºC; to prepare 10µM concentration as work primer suspended 10 pmol/µl in 90 µl of free DNase/RNase to reach a final concentration 10µM.

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REFERENCES

Abdelsalam A, Rashed L, Salman T, Hammad L and Sabry D (2016) Molecular assessment of Vitamin D receptor polymorphism as a valid predictor to the response of Interferon/Ribavirin based therapy in Egyptian patients with Chronic Hepatitis C. *Journal of Digestive Diseases* **17**(8), 547–553.

Abeer F Muraad, Ammar S Khamis, Ali H Al-Marzoqi, Ammar A Shalan, Zahraa M Al-Taee, ZainabHaider Al-Mosawi, Lina Hassan Alasady, Worood Hamza Aldahmushe, Worood Alwan Al- Jeafry and Shereen Sabah (2018) Molecular Study for association of ABCB1 gene Polymorphism with Leukemia patients. *Journal of Global Pharma Technology* **10**, 9-10.

Ali Hussein Al-Marzoqi, Nisreen Kaddim Radi and Ammal Raqib Shamran (2015) Human genetic variation susceptibility associated with HBV: analysis of genetic markers susceptibility to hepatitis b among patients in babylon province. *World Journal of Pharmaceutical Research* **4**(4), 102-113.

Ali Hussein Al-Marzoqi (2017) Incidence of EBV and HTLV-1 Infection Associated With Leukemia Patients. *Journal of Global Pharma Technology* **12**(9), 147-151.

Ali Hussein Al-Marzoqi (2018) Microbiological and molecular investigation of virulence factors for klebsiellapneumoniae liver abscess (KPLA). *Biochem. Cell. Arch.* **18**(1), 37-43.

Ammar S Khamis, Lena Fadhil Al-Jibouri, Ali H Al-Marzoqi, Ammar A Shalan, Zahraa M Al-Taee, Shahad F AL Morshdi, Ragheda A Al Hajam, Duaa H and Khuld W Al Dulamy (2018) *Helicobacter pylori* Genotype as Predicts Risk of (Ulcer Disease, Gastric Cancer, Non-Ulcer Dyspepsia); Role of some genes Mediated Signaling in infection. *Journal of Pharmaceutical Sciences and Research* **V10**/No. (8-9).

Arai T, Atsukawa M, Tsubota A, Kondo C, Shimada N, Abe H, Itokawa N, Nakagawa A, Okubo T and Aizawa Y (2015) Vitamin D related gene polymorphisms do not influence the outcome and serum vitamin D level in pegylated interferon/ribavirin therapy combined with protease inhibitor for patients with genotype 1b chronic hepatitis C. *Journal of Medical Virology* **87**(11), 1904-1912.

Babbs C, Smith A, Hunt L P, Rowan B P, Haboubi N Y and Warnes T W (1988) Type III pro-collagen peptide: A marker activity and prognosis in primary biliary cirrhosis. *Lancet*. **1**, 1021–1024.

Beard J A, Bearden A and Striker R (2011) Vitamin D and the anti-viral state. *Journal of Clinical Virology* **50**(3), 194-200.

Bellamy R, Ruwende C, Corrah T, Mc Adam K P W J, Thursz M and Whittle H C (1999) Tuberculosis and chronic hepatitis B virus

infection in Africans and variation in the vitamin D receptor gene. *J Infect Dis.* **179**, 721–724.

Chu C M (2000) Natural history of chronic hepatitis B virus infection in adults with emphasis on the occurrence of cirrhosis and hepatocellular carcinoma. *J Gastroenterol Hepatol*. **15**, E25–E30.

Dalhoff K, Dancey J, Astrup L, Skovsgaard T, Hamberg K J and Lofts F J (2003) A phase II study of the vitamin D analogue Seocalcitol in patients with inoperable hepatocellular carcinoma. *Br J Cancer* **89**, 252–257.

Edlin B R, Eckhardt B J, Shu M A, Holmberg S D and Swan T (2015) Toward a more accurate estimate of the prevalence of hepatitis C in the United States. *Hepatology* **62**, 1353–1363.

El Husseiny N M, Fahmy H M, Mohamed W A and Amin H H (2012) Relationship between vitamin D and IL-23, IL-17 and macrophage chemoattractant protein-1 as markers of fibrosis in hepatitis C virus Egyptians. *World Journal of Hepatology* **4**(8), 242–247.

Fan L, Tu X, Zhu Y, Zhou L, Pfeiffer T, Feltens R, Stoecker W and Zhong R (2005) Genetic association of vitamin D receptor polymorphisms with autoimmune hepatitis and primary biliary cirrhosis in the Chinese. *J Gastroenterol Hepatol*. **20**, 249–255.

Falleti E, Bitetto D, Fabris C, Cussigh A, Fontanini E, Fornasiere E, Fumolo E, Bignulin S, Cmet S and Minisini R (2010) Vitamin D receptor gene polymorphisms and hepatocellular carcinoma in alcoholic cirrhosis. *World J Gastroenterol.* **16**, 3016–3024.

Farid S, Rashed L and Sweilam S (2016) The Role of Vitamin D during Therapy in Chronic Hepatitis C Virus Infection and Its Relation to CYP 27 B1-1260 Promoter Polymorphism. *Egyptian Journal of Hospital Medicine* **64**, 287-303.

Gal Tanamy M, Bachmetov L, Ravid A, Koren R, Erman A, Tur Kaspa R and Zemel R (2011) Vitamin D: an innate antiviral agent suppressing hepatitis C virus in human hepatocytes. *Hepatology* **54**(5), 1570-1579.

Hewison M (2010) Vitamin D and innate and adaptive immunity. *Vitamins and hormones* **86**, 23-62.

Haussler M R, Whitfield G K, Haussler C A, Hsieh J C, Thompson P D and Selznick S H (1998) The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J Bone Miner Res*. **13**, 325–349.

Huang Y W, Liao Y T, Chen W, Chen C L, Hu J T, Liu C J, Lai M Y, Chen P J, Chen D S and Yang S S (2010) Vitamin D receptor gene polymorphismsand distinct clinical phenotypes of hepatitis B carriers in Taiwan. *Genes Immun*. **11**, 87–93.

Haussler M R, Whitfield G K, Haussler C A, Hsieh J C, Thompson P D and Selznick S H (1998) The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J Bone Miner Res*. **13**, 325–349.

Hussein O M Al-Dahmoshi, Ali H Al-Marzoqi and Israa H Noor (2018) Serum Levels of CD28 and CCL17 as a Markers for Immune Status and Progression of Leukemia in Babylon Province-Iraq. *P J M H S* **12**(1), 500-502.

Israa Hussein Noor Hassan, Ali Hussein Al-Marzoqi and Zainab Wahab Maroof (2016) Pharmacogenetics and Molecular study on BCR/ABL Inhibitors connected with Chronic Myeloid Leukemia; Genotype variability polymorphism among Babylon population. *Journal of Chemical and Pharmaceutical Research* **8**(7), 220-224.

Israa Hussein Noor Hassan, Ali Hussein Al-Marzoqi and Zainab Wahab Maroof (2016) Incidences of glutathione-S transferase genotypes among Iraqi patients associated with chronic myeloid leukemia. *International Journal of PharmTech Research* **9**(8), 166-173.

Israa Hussein Noor Hassan, Ali Hussein Al-Marzoqi and Zainab Wahab Maroof (2016) Influence of CYP3A5 gene polymorphisms and risk of chronic myeloid leukemia. *International Journal of Pharm Tech. Research* **9**(12), 634-639.

Israa Adnan Ibraheem, Hawraa Jawad Kadum and Ali Hussein Al-Marzoqi (2018) Molecular study for the role and prognostic significance of some cytokines among iraqi patients with hepatitis c tgf-b1-509 and ifn-g g-201a snps. *Biochem. Cell. Arch.* **18**(1), 271-277.

Kamal S M (2008) Acute hepatitis C: a systematic review. *Am J Gastroenterol.* **103**.

Kamen D L and Tangpricha V (2010) Vitamin D and molecular actions on the immune system: Modulation of innate and autoimmunity. *J. Mol. Med.* **88**, 441-450.

Lange C M, Miki D, Ochi H, Nischalke H D, Bojunga J, Bibert S, Morikawa K, Gouttenoire J, Cerny A and Dufour J F (2013) Genetic analyses reveal a role for vitamin D insufficiency in HCV-associated hepatocellular carcinoma development. *PLoS One* **8**, e64053.

Lena Fadhil, Ali Malik Saad, Ali Hussein Al-Marzoqi, Ammar A Shalan, Zahraa Mohammad Al Taee and Nebras Mohammed Sahi (2017) Genetic Study for the Role and Predictive Consequence of Certain Cytokines in Hepatitis B Iraqi Patients. *Journal of Global Pharma Technology* **09**(9), 75-83.

Li Y J, Tang Y W, Shi Y Q, Han S, Wang J B, Zhou X M, Chen Y, Wu Z D, Han Z Y and Han Y (2014) Polymorphisms in the vitamin D receptor gene and risk of primary biliary cirrhosis: A meta analysis. *Journal of Gastroenterology and Hepatology* **29**(4), 706-715.

Ly K N (2012) The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. *Ann Intern Med.* **156**, 271–278.

Matsumura T, Kato T, Sugiyama N, Tasaka Fujita M, Murayama A, Masaki T, Wakita T and Imawari M (2012) 25 hydroxyvitamin D3 suppresses hepatitis C virus production. *Hepatology* **56**(4), 1231-1239.

Petta S, Camma C, Scazzone C, Tripodo C, Di Marco V, Bono A, Cabibi D, Licata G, Porcasi R, Marchesini G and Craxý A (2010) Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. *Hepatology* **51**, 1158–1167.

Pourgholami M H, Akhter J, Ku Y and Morris D L (2000) *In vitro* and *in vivo* inhibition of liver cancer cells by 1, 25-dihydroxyvitamin D3. *Cancer Lett.* **151**, 97–102.

Pourgholami M H and Morris D L (2004) 1, 25-dihydroxyvitamin D3 in lipiodol for the treatment of hepatocellular carcinoma: cellular, animal and clinical studies. *J Steroid Biochem Mol. Biol*. **89–90**, 513–518.

Uitterlinden A G, Fang Y, Van Meurs J B, Pols H A and Van Leeuwen J P (2004) Genetics and biology of vitamin D receptor polymorphisms. *Gene* **338**, 143–156.

Sambrook J and Maniatis T (1989) Molecular cloning: a laboratory manual. Second edition. New York: Cold Spring Harbor Laboratory Press.

Sambrook J and Rusell (2001) Molecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Suneetha P V, Sarin S K, Goyal A, Kumar G T, Shukla D K and Hissar S (2006) Association between vitamin D receptor, CCR5, TNF-α and TNF-β gene polymorphisms and HBV infection and severity of liver disease. *J Hepatol*. **44**, 856–863.

Uitterlinden A G, Fang Y, van Meurs J B J, Pols H A P and van Leeuwen J P T M (2004) Genetics and biology of vitamin D receptor polymorphisms. *Gene* **338**, 143–156.

Valdivielso J M and Fernandez E (2006) Vitamin D receptor polymorphisms and diseases. *Clin. Chim. Acta* **371**, 1–12.

Villar L M, Del Campo J A, Ranchal I, Lampe E and Gomez M R (2013) Association between vitamin D and hepatitis C virus infection: a meta-analysis **19**(35), 5917-5924.

Veldman C M, Cantorna M T and DeLuca H F (2000) Expression of 1,25-dihydroxyvitamin D3 receptor in the immune system. *Arch Biochem. Biophys* **374**, 334–338.

Vogel A, Strassburg C P and Manns M P (2002) Genetic association of vitaminD receptor polymorphisms with primary biliary cirrhosis and autoimmunehepatitis. *Hepatology* **35**, 126–131.

Vu D, Sakharkar P, Tellez-Corrales E, Shah T, Hutchinson I and Min D I (2013) Association of vitamin D binding protein polymorphism with long-term kidney allograft survival in Hispanic kidney transplant recipients. *Molecular Biology Reports* **40**(2), 933-939.

Wang T J, Zhang F, Richards J B, Kestenbaum B, van Meurs J B, Berry D, Kiel D P, Streeten E A, Ohlsson C and Koller D L (2010) Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet*. **376**, 180 –188.

Whitfield G K, Remus L S, Jurutka P W, Zitzer H and Oza A K (2001) Functionally relevant polymorphisms in human nuclear vitamin D receptor gene. *Mol Cell Endocrinol*. **177**, 145–159.

Yao X, Zeng H, Zhang G, Zhou W, Yan Q, Dai L and Wang X (2013) Theassociation between the VDR gene polymorphisms and susceptibility tohepatocellular carcinoma and the clinicopathological features in subjects infectedwith HBV. *Biomed Res Int*. 953974.