

# Dynamic role of IL-33, ST2 axis, SOD, leptin and nitric oxide in pathogenicity and disease progression in HBV chronic infection in Iraqi patients

Hala Fouad Kasim AL-Eqabi<sup>1</sup>, Sabah Abd AL- Hameid Abd AL- Rahman<sup>2</sup>, Hazima Mossa ALabassi<sup>3</sup>

<sup>1,2</sup> Mustansiriyah University, College of Science / Department of Biology/Iraq

<sup>3</sup> University of Baghdad /Iraq

Email:essa90hala@gmail.com

## Abstract

Chronic hepatitis B infection remains a major health problem worldwide. The objective of this study was to investigate the role of some physiological and immunological aspect in the pathogenicity of HBV patients with chronic infection, in addition to assess the correlation coefficient between the two aspect and the effect of each on the other and their impact on the progression of the disease. The study was conducted on two hundred blood sample were collected from each participants in this study, 150 Iraqi men patients with chronic hepatitis B with age average 25- 55 years, in addition to 50 blood sample for apparently healthy men individuals with the same average age was used as control group. The samples were collected during the period from January 2021 to January 2022. And this study was done in the Hepatology and Gastroenterology Teaching hospital in Baghdad. All samples in present study were examined using the Polymerase Chain Reaction (PCR) technique to detect HBV DNA through the amplification of a pathogen's specific region of the genome using specific primers. And we selected the positive sample only then determine the IgM and IgG concentration to detect the chronic infection. Immunological parameters, the result revealed a high significant elevation ( $P < 0.01$ ) in the mean value of IL-33 and IL-33R axis and their receptors for patients ( $236.60 \pm 14.41$ ), ( $268.84 \pm 17.92$ ) Pg/ml respectively ( $P \leq 0.01$ ), when compared to control group, ( $161.46 \pm 14.54$ ), ( $175.51 \pm 7.15$ ) Pg/ml respectively. physiological parameters, the result showed a high significant increase ( $P < 0.01$ ), the mean value of Leptin and nitric oxide levels for patients was ( $1.228 \pm 0.06$ ) ng/ml, ( $6.27 \pm 0.56$ ) IU/ml respectively ( $P \leq 0.01$ ), when compared to control group ( $0.919 \pm 0.06$ ) ng/ml, ( $2.919 \pm 0.21$ ) IU/ml respectively and a decrease in the level of SOD in patients ( $266.62 \pm 9.45$ ) Pg/ml when compared to control group ( $378.23 \pm 25.32$ ) IU/ml. According to the present result, we can conclude that the under studied immunological parameters may consider a good therapeutic target to prevent the disease progression and we can consider physiological parameters a good marker for disease progression.

**Keywords:** HBV, Chronic hepatitis B infection, IL-33, IL-33R, Leptin, nitric oxide, SOD

## INTRODUCTION

The Hepatitis B virus (HBV) is a major public health concern around the world (1). HBV infects one-third of the world's population (more than two billion people), posing a serious health threat; nonetheless, with the exception of immunocompromised individuals, 95 % of adult hepatitis B patients recover with little or no clinical treatment (2).

**Address for correspondence:** Hala Fouad Kasim AL-Eqabi, Mustansiriyah University, College of Science / Department of Biology/Iraq, Email:essa90hala@gmail.com

Received date: 14 August 2022

Accepted: 22 September, 2022

Published: 07 October, 2022

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** pnrjournal@gmail.com

How to cite this article: AL-Eqabi K F H, AL- Rahman S A A, ALabassi M H, Dynamic role of IL-33, ST2 axis, SOD, leptin and nitric oxide in pathogenicity and disease progression in HBV chronic infection in Iraqi patients., J Pharm Negative Results 2022;13(4):258-263.

Access this article online

Quick Response Code:



Website:

www.pnrjournal.com

DOI:

10.47750/pnr.2022.13.04.031

HBV can cause liver cirrhosis and hepatocellular cancer in the long run (3). The frequency of HBV in the general population varies by nation, from 1% in wealthy nations to roughly 8% in poor ones (4). According to study in Iraq found that the Maysan, Dhi Qar, Al Qadisiyah, and Al Muthanna had the lowest prevalence of positive HBs antigen (0.5%). The areas with the highest prevalence rates (>3%) were An Najaf, Dahuk, Salah ad Din, and Babil. Anti-HBs antibody prevalence peaked in the third decade of life (12.2%) and is marginally, but not significantly, higher as people get older. The first ten years of life have the highest prevalence of positive anti-HBs antibodies (32.2%). The risk of testing positive is dramatically elevated by 38% in the second decade of life and by 2.63 times in the first decade of life as compared to the third decade of life (5).

Cytokines have been widely used as immunomodulatory agents to regulate immune responses during CHB treatment, and facilitate communication among cells of both the innate and adaptive immune systems, they are essential regulators of inflammations and share in plenty of biological processes and related with immune challenges to respond to infections (6).

The diagnosis of hepatitis B is mostly made based on the finding of serum HBV markers. Hepatitis B is brought on by hepatitis B viral infection. Some studies have shown a correlation between an increase in blood leptin levels in HBV infection and inflammatory cytokines, adipose tissues, and macrophage phagocytosis. Leptin also causes the production of inflammatory cells from macrophages such as TNF-, IL-6, and IL-12, which can accelerate liver necrosis and degeneration. Leptin, which is expressed in numerous organs including the liver, is crucial for inflammation and hepatic fibrogenesis in viral liver disorders. Leptin can cause proinflammatory reactions, lipid peroxidation (7). IL-33 is a multifunctional cytokine involved in various disease conditions (8) (9) (10). IL-33, through the receptor complex composed of ST2 and IL-1RaP, can activate the MAP-kinase and NF-kB signaling pathways and promote Th2 response and cytokine production" (11).

The physiology and pathology of the liver are significantly influenced by nitric oxide (NO) and its products. Certain patterns of the impact of nitric oxide on the pathophysiology and development of liver disorders are documented, despite its various and complex activities. (12). Superoxide dismutase (SOD) is a vital part of the antioxidant defense mechanism. SOD is the first and most crucial line of enzymatic defense against oxidative stress, especially oxygen free radicals, among the antioxidant defense mechanisms. (13).

## Aims of Study

This study, aimed to investigate the role of some physiological and immunological aspects in the pathogenicity of HBV patients with chronic infection, in addition to assess the correlation coefficient between the two aspects and the effect of each on the other and their impact on the progression of the disease.

## MATERIALS AND METHODS

### Study Design

The case – control study was carried out on (200) samples which include (150) men with chronic viral hepatitis infection aged (25-55) years who attended to the Hepatology and Gastroenterology Teaching Hospital (Medical city) in Baghdad, and apparently healthy control subjects with total number of (50) healthy volunteers with age match to the patients group. The study lasted from January/2021 to January/2022. The patients were divided into three groups according to age, group1(G1): between (25-35) years, group2 (G2): between (36-45) years and group3(G3): between (46-55) years. Also, the healthy was divided into three groups according to age, group1: between (25-35) years, group2: between (36-45) years and group3: between (46-55) years.

All samples were examined using the Polymerase Chain Reaction (PCR) technique to detect HBV DNA through the amplification of a pathogen's specific region of the genome using specific primers. Then using commercially-available ELISA kits (Cusabio U.S.A). The results read by a Microwell reader and compared in a parallel manner with controls; optical density read at 450 nm on an ELISA reader.

### Statistical Analysis:

The Statistical Analysis System- SAS (14). Program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) and T-test was used to significant compare between means in this study.

## RESULTS AND DISCUSSION

Serum level of IL-33 and IL-33R sample was collected from patients, controls then tested for the presence of IL-33 and their receptor by using ELISA. Table (1) shows the mean value of serum level for (IL-33) pg/ml and their receptor in patients and control group. The result showed, high significant increase ( $p < 0.01$ ) in the mean value of IL-33- and their receptor for patients ( $236.60 \pm 14.41$ ) pg/ml, ( $268.84 \pm 17.92$ ) pg/ml respectively ( $P \leq 0.01$ ). when compared to control group, ( $161.46 \pm 14.54$ ) pg/ml, ( $175.51 \pm 7.15$ ) pg/ml respectively.

**Table 1:** Comparison between patients and control groups in IL-33-ST2 axis.

Group	Mean ± SE	
	IL-33 P g/ml	IL-33R P g/ml
Patients	236.60 ±14.41	268.84 ±17.92
Control	161.46 ±14.54	175.51 ±7.15
T-test	44.64 **	41.084 **
P-value	0.0005	0.0001

\*\* (P≤0.01).

Interleukin-33 (IL-33) a member of the IL-1 family was formerly thought to promote type 2 immune responses by activating mast cells and T helper 2 (TH2) cells. It is now becoming increasingly clear that IL-33 also strongly activates innate lymphoid cells of group 2 (ILC2s), regulatory T (Treg) cells, TH1 cells, CD8+ T cells, and natural killer (NK) cells. Thus, IL-33 can contribute to tissue homeostasis and responses to environmental stimuli, as well as play a significant role in innate and adaptive immunity (15).

IL-33 is a multifunction cytokine involved in various disease condition (9). These results of serum IL-33 levels agreement with that reported by Wang *et al.*, (2012)(16), who studied the serum IL-33 levels its association with liver damage in China patients with chronic hepatitis B and found soluble ST2 (sST2) in CHB patients and healthy control were determined using enzyme-linked immunosorbent serologic assay, it was found that the levels of serum IL-33 in CHB patients were significantly higher than the healthy control at the base line. The levels of serum sST2 as a decoy receptor of IL-33 were significantly higher in CHB patients than the healthy control, These results suggest that IL-33 is involved in the pathogenesis of CHB. As well as other source have proven that the serum levels of IL-33 levels in patients with CHB were significantly higher than those in healthy controls. (17). Also Other study study in France at 2016 was demonstrated that the soluble IL-33 or sST2 can be employed as non-invasive diagnostic or prognostic biomarkers of liver disorders, according to the ELISA-based clinical data. However, the type of viral hepatitis, the liver pathology, or the stage of liver disease are all related to variations in serum IL-33/sST2 concentration levels. To officially recognize IL-33/sST2 as hepatitis biomarkers. (18). Also Other study in China at 2020 was demonstrated that, serum levels of IL-33 and its soluble receptor ST2 were

elevated in patients infected with chronic hepatitis B when compared with healthy controls. (19).

These results of serum IL-33 levels and sST2 levels agreement with that reported by Wei Yuan *et al.*, (2020) (20), who studied that the evaluation of the progression and mortality in CHB patients with hepatic flare might be done using the IL-33/sST2 axis and showed that patients in which the disease progressed to HBV-ACLF had the highest serum IL-33 and sST2 levels among the three groups (P<0.001). According to studies, IL-33-deficient mice showed more severe Con A liver damage than WT controls (21). Additionally, IL-33 directly protects hepatocytes by activating NF- $\kappa$ B, p38 MAPK, cyclin D1, and Bcl-2, which prevents liver damage (22). As was already established, IL-33 protects against liver damage and helps to reduce inflammation. This could be as a result of IL-33's capacity to target ST2, which is expressed on Treg cells and ILC2s (23).

#### The distribution of IL-33-ST2 axis in serum according to the age group.

Table (2) shows the distribution of IL-33-ST2 axis in serum of study groups distributed into three age groups: the first age group involved subjects with an age range (25-35) years, second age group involved sampled with an age range (36-45) years, the last group involved an age range (46-55) years. The result showed that, there were significant differences between IL-33 and studied age groups. The mean value of IL-33 in second studied group (36-45) years has different value at (293.35 ±31.52) pg/ml while, other age groups (25-35) years, (46-55) years have (223.61 ±11.25) pg/ml, (198.99 ±24.72) pg/ml respectively (P≤0.05). furthermore, no significant difference between studied age groups and IL-33R.

**Table 2:** Effect of Age groups in IL-33-ST2 axis of patients

Age Group (year)	Mean ± SE	
	IL-33 pg/ml	IL-33R pg/ml
25-35	223.61 ±11.25 b	241.38 ±20.94
36-45	293.35 ±31.52 a	285.31 ±40.74
46-55	198.99 ±24.72 b	279.33 ±30.79
LSD value	65.57 *	16.57 NS
P-value	0.0196	0.544

Means having with the different letters in same column differed significantly. \*, NS: Non-Significant.

Our study does not agree with the study of Aqbal, (2020) (24), which showed the highest frequency of IL33 level in the age group between (36 -45) year the mean was 36.04 pg/ml, followed by the age group between (15-25) year was 35.51pg/ml, then the age group between (46 -55) year was 31.37 pg/ml, and finally, the age group between (26-35) year was 31.06 pg/ml. There were no significant differences in the distribution of age among the four age groups. Furthermore there are no studies correlate the effect age with HBV infection and the role of IL-33 neither in the references nor on the web.

### Super oxide dismutase (SOD), leptin and Nitric oxide parameters

Table (3) shows a highly significant ( $P < 0.0001$ ) decrease in SOD, nitric oxide concentration in the serum of patients of viral hepatitis B ( $266.62 \pm 9.45$ ) pg/ml ( $6.27 \pm 0.56$ ) pg/ml respectively when compared with control group ( $378.23 \pm 25.32$ ) pg/ml, ( $2.919 \pm 0.21$ ) pg/ml. Also in this table leptin hormone level showed highly significantly ( $P < 0.0015$ ) increased in patients of HBV group ( $1.228 \pm 0.06$ ) ng/ml in comparison with control group ( $0.919 \pm 0.06$ ) ng/ml

**Table 3:** Comparison between patients and control groups in Super oxide dismutase (SOD), leptin and Nitric oxide parameters

Group	Mean ± SE		
	SOD pg/ml	Leptin ng/ml	Nitric oxide IU/ml
Patients	$266.62 \pm 9.45$	$1.228 \pm 0.06$	$6.27 \pm 0.56$
Control	$378.23 \pm 25.32$	$0.919 \pm 0.06$	$2.919 \pm 0.21$
T-test	109.359 **	0.187 **	1.129 **
P-value	0.0001	0.0015	0.0001

\* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ), NS: Non-Significant.

these results are compatible with the result of Atheer *et al.*, (2013) (25), who showed that the SOD activity, Vit C, Vit E and albumin were significantly lower in patients with HBV when compared to control group, these study supposed that deficiency of antioxidant barrier may cause oxidative stress in patients with HBV, and may be antioxidant treatment showed be useful for these patients. On the other hand, Hosnie and Soudabeh (2020) (26), showed that the SOD activity was significantly ( $P < 0.01$ ) lower in HBV patients ( $125.05 \pm 55.55$ ) n/ml compared to healthy control ( $271 \pm 74.23$ ) n/ml, this can cause liver damage and aggravate the complications in hepatitis B patients, it is known that SOD plays an important role in the defense against oxidative stress.

HBV is a clinically common disease. Numerous studies have explored the relationship between serum leptin levels and HBV. High serum leptin levels were observed in HBV patients compared with control. Therefore, serum leptin levels may serve as prognostic marker for hepatitis B disease (27). In recent years, an increasing number of clinical trials have focused on the relationship between leptin and HBV

.As an inflammatory mediator, leptin can affect various physiological processes and immune responses and can mediate T cell immune to hepatitis (28). The formation of nitric oxide (NO) a free radical, increases in liver disease, where the L-arginine-nitric oxide (L-arg-NO) pathway is activated by the increased levels of cytokines and endotoxins.

Guldal Kirkali *et al.*, (2000) (29) studied the nitric oxide in chronic liver disease and found that the mean serum nitrite concentration in cirrhosis, chronic active hepatitis were increased in both patient groups ( $84.8 \pm 9.43$ )  $\mu\text{mol/L}$ , ( $72.79 \pm 6.9$ )  $\mu\text{mol/L}$ . These results were in agreement with the search result. Serum nitrate and nitrite concentration may be elevated in patients with chronic hepatitis, this increase may be due to enhanced cytokine expression, as mentioned by Simpson (1997) (30).

### SOD, Leptin, and Nitric oxide in different age of patients

As shown in (Table 4), superoxide dismutase (SOD) enzyme showed non significant between age groups of patients, but

the level of SOD decreased with increase of age ,SOD in patients age 25-35 years , 36-45 years and 46-55 years were ( 274.32 ±11.55) pg/ml,( 267.38 ±23.04) pg/ml and (234.58 ±1 4.30) pg/ml respectively. Furthermore in this table it was observed that the leptin and nitric oxide level increased

significantly (P<0.0768) (P<0.585) respectively with age 25-35 years,36-45 years and 46-55 years , it was (1.041 ±0.12, 1.225 ±0.12) ng/ml and (1.386 ±0.05) ng/ml , (3.64 ±0.37) ng/ml, (4.27 ±0.54) ng/ml , (4.38 ±0.58) ng/ml respectively.

**Table 4:** Effect of Age groups in SOD, Leptin, and Nitric oxide of patients

Age Group (year)	Mean ± SE SOD pg/ml	Leptin ng/ml	Nitric oxide IU/ml
25-35	274.32 ±11.55	1.041 ±0.12 a	3.64 ±0.37
36-45	267.38 ±23.04	1.225 ±0.12 b	4.27 ±0.54
46-55	234.58 ±14.30	1.386 ±0.05 ab	4.38 ±0.58
LSD value	46.49 NS	0.296 *	1.493 NS
P-value	0.690	0.0768	0.585

Means having with the different letters in same column differed significantly. \* (P≤0.05), NS: Non-Significant.

Among the antioxidant defense system, SOD is the most important antioxidant enzyme to prevent cellular injury from ROS (31).Therefore ,we hypothesize that SOD plays a significant role in protecting cells against severe oxidative stress, and thus ,preventing the occurrence of hepatocellular carcinoma (HCC). SOD plays acritical role in the alleviation of H2o2 that was found in RBC and because hemoglobin and SOD has been proven to be in closely linked in RBC in the present results agree with other studies that have shown increased SOD level (25). These results are compatible with the results of Manolakopoulos *et al.* (2007) (32), found a significant association between serum leptin and the stage of hepatic fibrosis was noted patients with cirrhosis presented higher serum leptin levels compared to those with lower fibrosis stage ,increased serum leptin levels represent a negative prognostic factor for response to lamivudine monotherapy in patients .From the study of Koulentaki *et*

*al.*,(2001) (33).HBV had a lower carrier rate under the age of 20 years and peak carrier rates in middle age groups. Moreover ,these study showed the relationship between serum leptin and age seemed to be linear in a large population which means the older age is the higher serum leptin concentration. Nitric oxide (NO) rate over the age of 46 years in HBV had lower level (2.44 ± 0.23 )IU/ml and peak rates in middle age 36-45 years groups (3.41± 0.53) IU/ml and the middle level was at the lowest age (25-35) years in the research (2.86 ± 0.28)IU/ml .However there was no significant between three age groups ,this results indicates that the age does not play a role here , but depends on cirrhosis of the liver . A possible explanation that difference in the severity of inflammation and fibrosis may cause varying serum NO levels (29). Also ,the etiology of chronic hepatitis may explain the discrepancy between some previously reported studies and our results.

**Table 5:** Correlation coefficient between Physiological and Immunological parameters of patients

Parameters	Correlation coefficient-r	
	IL-33	IL-33R
SOD	-0.32 *	-0.30 *
Leptin	0.18 NS	0.21 NS
Nitric oxide	-0.37 *	-0.23 NS

\* (P≤0.05), NS: Non-Significant.

the result showed that a significant correlation between SOD and IL-33 was (0.32) these result agreement with the study of Stankovic *et al.*,(2016)(34) who showed that IL-33/ST2 axis plays a role in enhancing inflammation and tissue damage at the site of acute inflammation by affecting the concentration of magnesium and GSH important for antioxidative capacity ,as well as gene expression of anti-inflammatory cytokine TGF-beta . IL-33,inturn ,act on macrophages to induce microbial nitric oxide release .This induction was dependent on inducible nitric oxide synthesis (i NOS) activation .( 35) . Further ,there are no significant between leptin and IL-33-ST2 axis in patients.

## CONCLUSION

According to findings of present study, The physiological parameters (Leptin and SOD, differed in the three age groups. And the immunological parameters (IL-33 , IL-33R) of patients were highly significant increased compared to control group .This change in the indicators of immune parameters in the body may cause a change in liver function or liver damage .further The effect of the three different groups age on the immunological and physiological parameters not related to the age .This indicates that age has no impact on the disease ,but the stage of the disease has a strong impact on the immunological and physiological parameters.

## REFERENCES

- World health organization: WHO hepatitis B.htm. WHO, Geneva, Switzerland. <http://www.WHOhepatitisB.htm>. Accessed on 22 June 2015.
- World Health Organization.2019. Hepatitis B. Available online: <https://www.who.int/news-room/fact-sheets/detail/hepatitis-b>
- Mehta, P.; Reddivari, A.K.R. Hepatitis; StatPearls Publishing: Treasure Island, FL, USA, 2021. [PubMed]
- Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of agespecific antibody to HCV sero-prevalence. *Hepatology* 2013; 57: 1333–1342. [PubMed] [Google Scholar]
- Alsamarai AM, Abdulrazaq G, Fatah A, Alobaidi AHA (2016) Seroprevalence of Hepatitis B Virus in Iraqi Population. *J Vaccines Immunol* 2016: J102.
- Masi, A., Glozier, N., Dale, R. and Guastella, A.J., 2017. The immune system, References 88 cytokines, and biomarkers in autism spectrum disorder. *Neuroscience bulletin*, 33(2), pp.194-204.
- Şenol A, Toraman Z, A.(2021). The Importance of Serum Leptin Level in Chronic Viral Hepatitis B and C. *Aegean J Med Sci* 2021;1:14 -19.
- Barksby HE, Lea SR, Preshaw PM, Taylor JJ. 2007. The expanding family of interleukin-1 cytokines and their role in destructive inflammatory disorders. *Clin Exp Immunol* 149:17–25.
- Liew FY, Pitman NI, McInnes IB. 2010. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol* 10:103–110. Review.
- Mok MY, Huang FP, Ip WK, Lo Y, Wong FY, Chan EY, Lam KF, Xu D. 2010. Serum levels of IL-33 and soluble ST2 and their association with disease activity in systemic lupus erythematosus. *Rheumatology* 49:520–527.
- Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA. 2005. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 23:479–484
- Iwakiri Y, Kim MY. *Trends Pharmacol Sci*. 2015 Aug;36(8):524-36. doi: 10.1016/j.tips.2015.05.001. Epub 2015 May 28. PMID: 26027855 Free PMC article. Review.
- Zhang X, Lu Y, Rong C, Yang D, Li S, Qin X. Role of superoxide dismutase in hepatitis B virus-related hepatocellular carcinoma. *J Res Med Sci* 2016;21:87
- SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
- Liew FY, Girard JP, Turnquist HR.(2016). Interleukin-33 in health and disease. *Nat Rev Immunol*. Nov;16(11):676-689. doi: 10.1038/nri.2016.95. Epub 2016 Sep 19. PMID: 27640624 Review.
- Wang, J, Cai Y, Ji, H, Junyan Feng J, Ayana DA, Junqi Niu J, and Jiang Y.( Serum IL-33 Levels Are Associated with Liver Damage in Patients with Chronic Hepatitis B. *Journal of interferon & cytokine research* Volume 32, Number 6, 2012.
- Huan S, Zhao J, Wang Z, Gao S and Wang K. Relevance of serum interleukin-33 and ST2 levels and the natural course of chronic hepatitis B virus infection. Huan *et al. BMC Infectious Diseases* (2016) 16:200
- Arshad MI, Khan HA, Piquet-Pellorce C, Samson M (2016) Interleukin-33 and its Soluble Receptor ST2 as Non-Invasive Biomarkers of Human Hepatitis. *JSM Hepat* 1(1): 1003.
- Gao X, Chi X, Wang X, Wu R, Xu H, Zhan M, Li D, Ding Y, Xu D, Niu J. *Mediators Inflamm*. 2020 Apr 28;2020:1403163. doi: 10.1155/2020/1403163. E Collection 2020. PMID: 32410845 Free PMC article.
- WeiYuanMM..XueMeiMM. Yu-yiZhangMM. Zheng-guo.ZhangMM. YingZouMM.HuiZhuMM.YuLiuMM.Jie-feiWangMM.Zhi-pingQianMM.Hong-zhouLu.(2020). High Expression of Interleukin-33/ST2 Predicts the Progression and Poor Prognosis in Chronic Hepatitis B Patients with Hepatic Flare. *The American Journal of the Medical Sciences*, Volume 360, Issue 6, December 2020, Pages 656-661
- M. I. Arshad, C. Piquet-Pellorce, A. L'Helgoualch. "TRAIL but not FasL and TNF $\alpha$ , regulates IL-33 expression in murine hepatocytes during acute hepatitis," *Hepatology*, vol. 56, no. 6, pp. 2353–2362, 2012.
- N. Sakai, H. van Sweringen, R. C. Quillin *et al.*, "Interleukin-33 is hepatoprotective during liver ischemia/reperfusion in mice," *Hepatology*, vol. 56, no. 4, pp. 1468–1478, 2012.
- H. R. Turnquist, Z. Zhao, B. R. Rosborough. "IL-33 expands suppressive CD11b+ Gr-1(int) and regulatory T cells, including ST2L+ Foxp3+ cells, and mediates regulatory T cell-dependent promotion of cardiac allograft survival," *Journal of Immunology*, vol. 187, no. 9, pp. 4598–4610, 2011
- Aqbal Suhail Najm (2020).The Role of IL-33 and IL-17 in viral Hepatitis B Patients.M.SC.Thesis.Mustansiriyah Univ.College of Sci.Biology Dep.Baghdad .Iraq.
- Atheer A.,Wesen A.and Amani M. jasim "Study Several Biochemical Parameters into patients with Hepatitis B virus ",*Global Journal of Medical research Disease* ,13 (2),1 (2013).
- Hosni Hoseini, Soudabeh Etemadi.(2020).Evaluation of Redox Status in patients with Hepatitis B Virus in Zahedan,Iran .*Journal of Clinical and Basic Research* .JCBR.4.3.10.ISSN:2538-3736.DOI:10.29252
- Qiong-yuan Zhang,Xuan XU,Mwi LUO,Jian-jiang XUE and Ying-LI.(2017).Serum Leptin Level in Patients with Hepatitis B:A Meta analysis.ABSR,vol.6. DOI, <https://doi.org/10.2991/bst-17.2018.1>
- Fantuzzi G., Adipose tissue ,adipokines ,and inflammation .*J Allergy clin Immunol*,2005.115(5):p.911-9;quiz 920.
- Guldal Kirkali,Semra Gezer,Nurcan Umur,Mehmet Ali Ozcan,Ethem Tankurt.(2000).Nitric Oxid in Chronic Liver Disease.*Turk J Med Sci* 30(2000) 511-515
- Simpson KJ.Lukacs NW.Coletti L,Strieter RM.KunkelSL.Cytokines and liver.*J Hepatol* 27:1120-32.1997
- Choi EJ, Kim GH. Antioxidant and anticancer activity of *Artemisia princeps* var. *orientalis* extract in HepG2 and Hep3B hepatocellular carcinoma cells. *Chin J Cancer Res* 2013;25:536-43.
- Manolakopoulos G, Bethanis S, Liapi C, Stripeli F, Sklavos P, Margeli A, Christidou A, Katsanika A, Vogiatzakis E, Tzourmakliotis D, Theocharis S. *BMC Gastroenterol*. 2007 May 31;7:17. doi: 10.1186/1471-230X-7-17. PMID: 17540037
- Koulantaki M, Ergazaki M, Moschandreia J,Spanoudakis S,Tzagarakis N,Drandakis PE.Prevalence of hepatitis B and C markers in high-risk hospitalised patients in Crete :a five -year observational study .*BMC Public Health* 2001;1:17-24.Epub 2001 Dec 21.
- Stankovic MS, Janjetovic K, Velimirovic M, Milenkovic M, Stojkovic T, Puskas N, Zaletel I, De Luka SR, Jankovic S, Stefanovic S, Japundzic-Zigon N, Petronijevic ND, Trajkovic V, Trbovich AM.*Exp Mol Pathol*. 2016 Aug;101(1):31-7. doi: 10.1016/j.yexmp.2016.05.012. Epub 2016 May 21. PMID: 27222019
- Li C, Li H, Jiang Z, Zhang T, Wang Y. (2014) Interleukin-33 Increases Antibacterial Defense by Activation of Inducible Nitric Oxide Synthase in Skin. *PLoS Pathog* 10(2): e1003918. doi:10.1371/journal.ppat.1003918