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Original Article

Tumor necrosis factor-alpha and insulin resistance in chronic viral hepatitis B in Makurdi, Nigeria

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Abstract

Background and Aim: Previous studies have reported manifestations other than liver disease in patients with chronic hepatitis B (CHB), with specific reports of incidental type 2 diabetes mellitus. This study aimed at determining the impact of CHB disease on insulin resistance and its correlation with tumor necrosis factor-alpha (TNF-α).

Methods: Seventy CHB patients aged 18–55 years attending a tertiary hospital were selected as study group subjects and anthropometrically matched with 65 apparently healthy controls. Blood pressure (BP), body mass index (BMI), TNF-alpha, fasting serum insulin (FSI), fasting plasma glucose (FPG) and homeostasis model assessment of insulin resistance (HOMA-IR) were measured in both study and control group subjects.

Results: There was no significant ($P > 0.05$) difference between the mean systolic blood pressure (BP), diastolic BP, age, and body mass index of CHB and controls. There was a significant ($P < 0.05$) increase in TNF-α, fasting plasma glucose, and homeostasis model assessment of insulin resistance (HOMA-IR) in CHB than the controls. There was a significant ($P < 0.01$) direct correlation between TNF-α and HOMA-IR ($r = 0.534$), TNF-α and fasting serum insulin ($r = 0.509$) in CHB patients.

Conclusion: CHB infection induces insulin resistance with TNF-α mediating the insulin resistance. In terms of effective management, CHB patients may need to be monitored for the occurrence of insulin resistance and diabetes mellitus.

Key words: Chronic hepatitis B, insulin resistance, tumor necrosis factor-alpha

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INTRODUCTION

Impaired action of insulin at its main target organs is referred to as insulin resistance, known as the major inducer of metabolic syndrome and type 2 diabetes mellitus (T2DM).[1,2] Cellular insulin signaling is mediated by complex, highly integrated network molecules. In response to insulin stimulation, insulin receptor phosphorylates insulin receptor substrate (IRS) proteins, which are linked to the activation of two main signaling pathways: The phosphatidylinositol 3-kinase–Akt/protein kinase B pathway, which is responsible for the majority of the metabolic actions of insulin, and the Ras–mitogen-activated protein kinase pathway, which control cell growth and differentiation.[3,4] Pathogenesis of insulin resistance has been shown to be due to impairment of insulin signaling, predominantly mediated by regulators or modulators of insulin receptor function.[5]

Two billion people in the world are estimated to be infected with hepatitis B virus (HBV),[6] of this number an estimated 350 million (representing 17.5% of the 2 billion) worldwide are chronically infected with HBV.[7] About 600,000 deaths occur yearly due to the consequences of acute or chronic HBV.[8] Individuals who fail to spontaneously or naturally clear the virus after acute infection become chronic carriers of the

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viruses.[9,10] Chronicity to hepatitis B viral infection has been defined as continuously testing positive to hepatitis B surface antigen (HBsAg) for 6 months or longer.[10,11] Chronic hepatitis B (CHB) infection has been linked with extrahepatic clinical manifestations.[12,13] Previous survey studies in CHB revealed incident T2DM.[14-17]

Tumor necrosis factor-alpha (TNF-α) is a cytokine with many functions. Overexpression of proinflammatory cytokines has been reported to be stimulated by HBV.[18,19] Hotamisligil *et al*. in 1993 reported the first evidence of a link between TNF-α and insulin resistance.[20] Their study revealed that obese animal's adipocytes expressed increased TNF-α level which caused insulin resistance. Subsequent studies by Serino *et al.* also supported a link between TNF-α and insulin resistance.[21] The neutralization of TNF-α with soluble receptors followed by an improvement in insulin resistance in animals and humans confirms the association of TNF-α and insulin resistance.[20,21] Therefore, this study is aimed at examining the impact of CHB disease on insulin resistance and its correlation with TNF-α in this infection.

MATERIALS AND METHODS

Selection of subjects

The case–control study was conducted in a tertiary hospital Makurdi, Nigeria. Ethical approval was obtained from the institutional ethics committee and the study was carried out within a period of 6 months. Written informed consent was sought from the individual patient by educating them on the need and relevance of the study. A structured questionnaire was administered to the consented patients, who answered the questions and returned same. The biochemical indices of randomly selected 70 CHB patients and 65 anthropometrically matched healthy controls aged 18–55 years were compared in the study.

Inclusion criteria

- Viral hepatitis B patients, who were continuously tested positive for HBsAg for up to 1 year during their periodic visit to the clinic, as chronic HBV patients
- Apparently healthy individuals with desired blood pressure (BP) and anthropometric indices (body mass index [BMI], age) seronegative for HBsAg and anti-hepatitis C virus (HCV) antibody, as controls.

Exclusion criteria

- Individuals with conditions that predispose to elevated insulin resistance and TNF-α
- Patients on drugs that affect glucose metabolism
- HIV/AIDS patients
- Patients with malignancy and those unwilling to participate in the study were excluded from the study.

Anthropometric measurements

Height and waist circumference were noted using a measuring tape (to the nearest 0.1 cm), with the individuals wearing light clothes and no shoes. Waist circumference was measured at the midpoint between lower border of the rib cage and the iliac crest. Weight was measured to the nearest 0.1 kg using a mechanical weighing machine. BMI, defined as mass in kilograms divided by the square of height in meters, was calculated.

Sample collection and processing

Fasting venous blood samples were collected from the selected individuals. Six milliliters of blood was drawn from each individual aseptically and dispensed as follows; 2 ml of whole blood into fluoride oxalate bottles and plasma extracted was used for estimation of fasting plasma glucose (FPG). Four milliliters of the whole blood was dispensed into plain tubes and centrifuged after clot retraction. The serum was extracted aseptically and stored at -70 C for estimation of TNF-alpha using enzyme-linked immunosorbent assay (ELISA) and at -20 C for estimation of serum insulin by ELISA technique. The serum was also used for the analysis of HBsAg, serum total protein, albumin, alkaline phosphatase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

Determination of blood glucose

The reagent kit for the determination of plasma glucose was obtained from Randox Laboratories Limited, United Kingdom. The Barham and Trinder glucose oxidase method was used in the determination of plasma glucose.[22] Glucose in the sample (standard, control, and plasma) is oxidized by glucose oxidase in the reagent to yield gluconic acid and hydrogen peroxide. Hydrogen peroxide is broken down into water and oxygen; the oxygen released oxidizes the colorless para-aminophenazone in the presence of phenol in the reagent to yield a pink colored solution. The intensity of this pink colored solution is directly proportional to the concentration of glucose in the sample. The concentration of glucose in the plasma of the patients was determined using the formula:

Absorbance of serum solution/Absorbance of standard solution × Conc. of Std. (5.55 mmol/l).

Determination of serum tumor necrosis factor-alpha

The TNF-α immunoassay kit, obtained from DRG international California, USA, was used for the assay of TNF-α. The determination of TNF-α in serum was

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based on the ELISA sandwich principle. Monoclonal antibodies (MAbs) directed against distinct epitopes of TNF-α bind to TNF-α molecule. The capture MAb 1 immobilized onto the microtiter well binds TNF-α molecule. The addition of a detection MAb 2 labeled with horseradish peroxidase (HRP) binds the available antigenic sites on the TNF-α molecule. The TNF-α molecule is sandwiched between the two MAbs. The amount of binding of the detector antibody depends on the available antigenic sites of TNF-α molecule. The amount of the detector antibody bound to the TNF-α molecule is proportional to the amount of TNF-α molecule in the sample. The addition of a substrate to the enzyme mixture gives rise to products, which react with the chromogen tetramethylbenzidine (TMB) to yield a colored solution. The intensity of this colored solution is proportional to the concentration of TNF-α in the sample. The concentrations of TNF-α in the samples were determined by extrapolating the absorbances of the test against the concentrations on the standard curve plotted from the absorbances and concentration of the standards.

Determination of serum insulin

The insulin ELISA kit obtained from DRG international California, USA, was used in determining serum insulin. The determination of insulin was based on the ELISA sandwich principle where a MAb (capture antibody) immobilized onto the surface of microtiter wells is directed towards unique antigenic sites on the insulin molecule in the sample. A second anti-insulin antibody labeled (detection antibody) with HRP enzyme when added to the mixture binds to the available antigenic sites on the insulin molecule. The insulin molecule is sandwiched between the two antibodies. The amount of the detection antibody bound to the available antigenic sites is directly proportional to the amount of insulin present in the sample. Upon substrate addition, the products formed by the enzyme activity will be proportional to the enzyme bound to the insulin molecule. The product formed reacts with the chromogen TMB producing a colored solution whose intensity is directly proportional to the amount of insulin in the sample. The concentrations of insulin in the samples were determined by extrapolating the absorbances of the test against the concentrations on the standard curve plotted from the absorbances and concentration of the standards.

Determination of hepatitis B surface antigen

The Monolisa HBsAg ultra ELISA kit, obtained from Bio-Rad Marnes-la-Coquette France, was used for the assay of HBsAg. The determination of HBsAg was based on the ELISA sandwich principle where immobilized mouse monoclonal anti-HBs antibodies bind HBsAg in sample. The amount of antigen bound depends on the amount in the sample. A second MAb peroxidase labeled is added to the mixture which binds the available epitopes on the HBsAg. The amount of the second MAb bound to the HBsAg is proportional to the amount of HBsAg. In addition to an enzyme substrate, a product is formed which reacts with TMB to yield a colored solution. The intensity of the solution is proportional to the concentration of the HBsAg present in the sample. The intensity of the color of the final solution was measured as the absorbances using a microplate reader set at a wavelength of 450 nm. The test results were calculated using a cutoff value determined by the formula: Cutoff (Co) = NC + 0.050 where NC denotes absorbance value of the negative control. Interpretation of the results follows: Cutoff value of ≤ an absorbance (OD) of 0.080 was interpreted negative and an absorbance (OD) value of ≥1.000 was interpreted positive.

Determination of total proteins

The reagent kit for the determination of total protein was obtained from Randox Laboratories Limited, United Kingdom. The Biuret end point method was used in determining serum total proteins. In this method, the peptide bonds of the protein molecule in the sample (standard, control and serum) interact with cupric ions in an alkaline medium in the Biuret reagent to form a purple color.[23] The intensity of the purple color is proportional to the concentration of total proteins in the sample. The intensity of the purple color obtained was spectrophotometrically measured at a wavelength of 540 nm to obtain the absorbances of the sample solutions. The concentration of protein in the serum of patients was determined using the formula:

Absorbance of serum solution/Absorbance of standard solution × Conc. of Std. (58.5 g/l).

Determination of serum albumin

The reagent kit for the determination of serum albumin was obtained from Randox Laboratories Limited, United Kingdom. Serum albumin was determined by the bromocresol green (BCG) end point method. The measurement of serum albumin is based on its quantitative binding to the indicator BCG. The intensity of the colored albumin-BCG-complex is proportional to the concentration of albumin in the sample.[24] The intensity of the deep green colored solution obtained was spectrophotometrically measured at a wavelength of 578 nm to obtain the absorbances of the serum, standard, and control solutions. The concentrations of albumin in these samples were determined using the formula:

Absorbance of serum solution/Absorbance of standard solution × Conc. of Std. (46.7 g/l).

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Determination of alanine and aspartate aminotransferases

The reagent kit for the determination of serum aminotransferase enzymes were obtained from Randox Laboratories Limited, United Kingdom. The aminotransferase enzymes were assayed using the colorimetric end point method. AST catalyses the transfer of an amino group from L-aspartate to α-oxoglutarate to form glutamate and oxaloacetate. ALT catalyses the transfer of an amino group from L-alanine to α-oxoglutarate to form glutamate and pyruvate. In the presence of 2, 4- dinitrophenyl hydrazine, the oxaloacetate (from AST) and pyruvate (from ALT) respectively produces oxaloacetate hydrazone and pyruvate hydrazone complex. This complex reacts with sodium hydroxide to form a red coloured solution, with an intensity that is directly proportional to the enzyme activity.[25] The absorbances of the final sample solutions were read against the reagent blank spectrophotometrically at a wavelength of 540 nm. The activities of the enzymes in the serum of patients were obtained by extrapolation of the absorbance's on a calibration graph.

Determination of alkaline phosphatase

The reagent kit for the determination of alkaline phosphatase was obtained from Quimica Clinica Aplicada S.A. Spain. Serum alkaline phosphatase was determined using the phenolphthalein monophosphate substrate end point method. The colorless phenolphthalein monophosphate substrate is hydrolyzed by serum alkaline phosphatase to phosphoric acid and phenolphthalein. The phenolphthalein in alkaline pH medium changes from colorless into a pink color. The intensity of this colored solution is directly proportional to the concentration of alkaline phosphatase in the sample.[26] The final colored solution which is stable for a minimum of 1 h was read spectrophotometrically at a wavelength of 550 nm and absorbances used to calculate the enzyme activity, using the formula:

Absorbance of serum solution/Absorbance of standard solution × Conc. of Std. (30 U/L).

Homeostatic model assessment method

Homeostatic model assessment (HOMA) method, which has been validated as a reliable measure of insulin sensitivity *in vivo* in humans, was used to estimate HOMA insulin resistance (HOMA IR). Insulin resistance was determined using a standardized Microsoft excel HOMA 2 calculator, based on Matthews *et al*.'s formula.[27]

Statistical analysis

The statistical package IBM Armonk, New York, United States SPSS version 21 was used in analyzing the data generated. Descriptive statistics were used in determining the mean and standard deviation of the parameters measured. The Student's *t*-test was used in comparing the mean of the parameters in CHB and control groups. Pearson correlation analyses was done to see the association between parameters measured in the viral hepatitis patients. Two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows the BP, age, BMI, TNF-α, fasting serum insulin (FSI), FPG, and HOMA-IR. There was no significant ($P > 0.05$) difference between the mean systolic BP, diastolic BP, age, and BMI of CHB and controls. There was a significantly ($P < 0.05$) elevated TNF-α, FPG, HOMA-IR in CHB than the controls.

Figures 1 and 2, respectively, show the correlation between TNF-α and HOMA-IR, TNF-α and FSI in CHB patients. There was a significant ($P < 0.01$) direct correlation between TNF-α and HOMA-IR ($r = 0.534$), TNF-α and FSI ($r = 0.509$) in CHB patients.

DISCUSSION

Besides HBV infecting and causing liver dysfunction, studies have reported extrahepatic manifestations of this disease. One of the extrahepatic manifestations of CHB virus (CHBV) has been proposed to be metabolic dysfunction, based on incidental T2DM reported in CHB infection. It is a well-known fact that insulin resistance mediates the development of metabolic syndrome and type 2 diabetes. There are studies showing that there is an association between insulin resistance and HBV disease. But there are also reports saying that there is no association between HBV disease and insulin resistance. In a bid to confirm the relationship between HBV and insulin resistance, our study measured insulin resistance along with TNF-α (a known independent mediator of insulin resistance) in CHBV. The study further determined the association between the immunological cytokine and insulin resistance to propose a possible cause–effect relationship in this viral disease. Advanced age and overweight are well-known principal inducers of insulin resistance along associated metabolic disturbances. To remove doubt cast on the results due to possible interferences from anthropometric indices, the CHBV patients were anthropometrically matched with the controls.

The result of our study showed increased levels of FPG, HOMA-IR, TNF-α, and FSI in CHBV patients compared with that of controls. However, the elevated FSI in CHBV compared with the controls was not statistically significant. The study also found a direct correlation

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Table 1: Blood pressure, anthropometric indices, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, tumor necrosis factor-alpha, insulin, fasting plasma glucose, homeostasis model assessment of insulin resistance in chronic hepatitis B virus and controls

Parameters	CHBV (*n*=70)	Control (*n*=65)	Critical *t* value	Calculated *t* value	*P*
Systolic BP (mmHg)	122.27±6.02	123.98±6.43	1.96	1.60	0.113
Diastolic BP (mmHg)	80.54±7.56	82.45±5.16	1.96	1.72	0.092
Age (years)	28.81±9.07	31.35±10.15	1.96	1.53	0.129
BMI (kg/m^2)	23.36±3.43	24.42±3.39	1.96	1.81	0.072
Total protein (g/l)	73.47±6.77	75.97±6.29	1.96	2.23	0.028
Albumin (g/l)	41.23±4.28	41.44±3.22	1.96	0.32	0.752
AST (U/L)	52.32±50.22	25.20±12.75	1.96	4.37	0.000
ALT (U/L)	30.75±39.06	10.56±9.60	1.96	4.19	0.000
ALP (U/L)	62.52±27.43	57.50±18.62	1.96	1.25	0.214
TNF-α (pg/ml)	19.48±23.06	13.08±13.37	1.96	1.99	0.047
Insulin (pmol/l)	76.76±67.76	59.89±39.72	1.96	1.78	0.077
FPG (mmol/l)	4.68±0.59	4.24±0.54	1.96	4.60	0.000
HOMA-IR	1.46±1.16	1.07±0.71	1.96	2.37	0.040

Data expressed are Mean±SD. Statistical analysis was done by Student's *t* test. *P*< 0.05 was considered significant. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, TNF-α: Tumor necrosis factor-alpha, BMI: Body mass index, FPG: Fasting plasma glucose, HOMA-IR: Homeostasis model assessment of insulin resistance, CHBV: Chronic hepatitis B virus, SD: Standard deviation, BP: Blood pressure

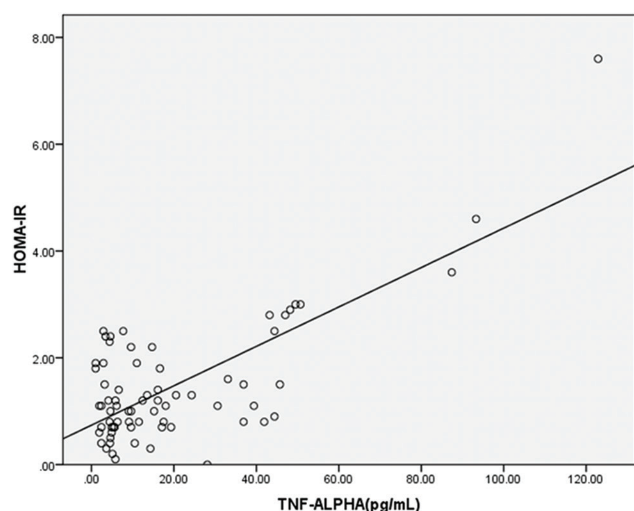

Figure 1: Correlation of tumor necrosis factor-alpha with homeostasis model assessment of insulin resistance in chronic hepatitis B virus patients

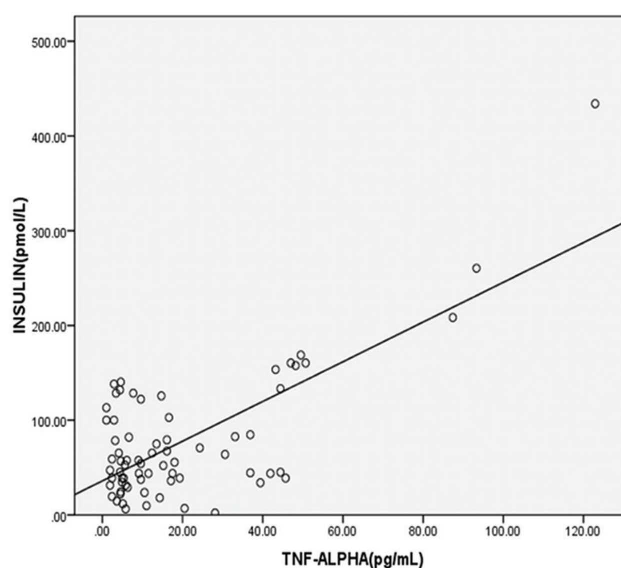

Figure 2: Correlation of tumor necrosis factor-alpha with insulin in chronic hepatitis B virus patients

between serum TNF-α level and IR, serum TNF-α level and FSI in CHBV. The hyperinsulinemia and higher fasting plasma glucose levels in CHBV could imply incident insulin resistance in CHBV disease. The positive correlation between TNF-α and IR implies, that elevated TNF-α could be the cause of the IR observed in the CHBV patients. The hyperinsulinemia and high insulin resistance observed in CHBV are consistent with the findings of Mohammad *et al.*, Kim *et al.*, Dai *et al.*, and Lee *et al.* who observed insulin resistance in CHBV infection.[16,28-31] On the contrary, another study reported that HBV carriers were not associated with insulin resistance.[32] The elevated TNF-α observed in CHBV is consistent with the finding of Kremsdorf *et al.*, who demonstrated the induction of synthesis of proinflammatory cytokines by HBV.[18]

The elevated FSI observed in the study indicates insulin-resistant state in CHBV patients. This hyperinsulinemia is compensatory, referred to as a positive feedback stimulation of the pancreatic beta cells to secret more insulin into circulation to meet the increasing demands of the body cells.[33] The mechanism of insulin resistance in CVHB infection remains unknown. Previous studies have reported HBx-induced hepatic steatosis and inflammation,[29,34,35] with a proposed negative effect on hepatic insulin signaling.[30] The HBx protein has been specifically shown in experimental studies to impair hepatic insulin signaling through degradation of IRS-1 and induction of suppressor of cytokine signaling-3 (SOCS-3), causing insulin resistance.[30] The HBx protein upregulates SOCS-3, which inhibits the tyrosine phosphorylation of

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IRS-1, thereby suppressing insulin-mediated glucose uptake.[30]

Steinberg *et al.* reported the impairment of insulin signaling in skeletal muscles mediated by TNF-α through TNF receptor. According to the report, TNF-α action stimulates the synthesis of protein phosphatase 2C, which inactivates activated AMP-protein kinase (AMPK).[36] The enzyme AMPK catalyzes the phosphorylation and activation of acetyl-CoA carboxylase (ACC). The enzyme ACC stimulates free fatty oxidation. Absence or inactivation of ACC results to elevated free fatty acids with the subsequent formation and accumulation of diacylglycerol mediating insulin resistance in skeletal muscles. In adipose tissues, TNF-α has been shown to suppress the expression of many intracellular proteins that are needed for insulin-mediated glucose uptake in adipocytes, such as insulin receptor, IRS-1, and glucose transporter 4 (GLUT4).[37] Among the first transcription factors shown to be targeted by TNF-α, signaling in adipocytes was the peroxisome proliferator-activated receptor gamma (PPARγ). TNF-α suppresses PPARγ activity: by inhibiting the expression of PPARγ mRNA or by the suppression of its transcriptional activity and by enhancing the phosphorylation of PPARγ.[37] TNF-α can suppress GLUT4 expression through suppression of PPARγ activity. TNF-α has also been shown to upregulate SOCS-3 gene in adipocytes causing adipocyte insulin resistance.[37]

CONCLUSION

Our study shows that CHB infection is associated with insulin resistance and TNF-α is proposed to be one of the mediators of insulin resistance. In terms of effective management, CHB patients may need to be monitored for the occurrence of insulin resistance and diabetes mellitus.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Eckel RH, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2010;375:181-3.
2. Grundy SM. Metabolic syndrome pandemic. Arterioscler Thromb Vasc Biol 2008;28:629-36.
3. Douglas MW, George J. Molecular mechanisms of insulin resistance in chronic hepatitis C. World J Gastroenterol 2009;15:4356-64.
4. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: Insights into insulin action. Nat Rev Mol Cell Biol 2006;7:85-96.
5. Bugianesi E. Hepatitis C virus and hepatic insulin resistance: Experimental evidence. Hot Top Viral Hepat 2009;14:11-5.
6. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-39.
7. Centers for Disease Control and Prevention. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States. Morb Mortal Wkly Rep 2005;54:1-32.
8. World Health Organization. Hepatitis B: WHO Fact Sheet NO204 Geneva, Switzerland; 2013:204.
9. Guidotti LG, Chisari FV. Immunobiology and pathogenesis of viral hepatitis. Annu Rev Pathol 2006;1:23-61.
10. World Health Organization. The immunological basis for immunization series of hepatitis B. Module 22 Geneva, Switzerland 2010;1-16.
11. Mast EE, Margolis HS, Fiore AE, Brink EW, Goldstein ST, Wang SA, *et al.* A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: Recommendations of the Advisory Committee on Immunization Practices (ACIP) part 1: Immunization of infants, children, and adolescents. MMWR Recomm Rep 2005;54:1-31.
12. Pagnoux C, Seror R, Henegar C, Mahr A, Cohen P, Le Guern V, *et al.* Clinical features and outcomes in 348 patients with polyarteritis nodosa: A systematic retrospective study of patients diagnosed between 1963 and 2005 and entered into the French Vasculitis Study Group Database. Arthritis Rheum 2010;62:616-26.
13. Reimold A. Viruses and arthritis: New challenges in diagnosis, therapy, and immunization. Am J Med Sci 2010;339:549-56.
14. Custro N, Carroccio A, Ganci A, Scafidi V, Campagna P, Di Prima L, *et al.* Glycemic homeostasis in chronic viral hepatitis and liver cirrhosis. Diabetes Metab 2001;27(4 Pt 1):476-81.
15. Li-Ng M, Tropp S, Danoff A, Bini EJ. Association between chronic hepatitis B virus infection and diabetes among Asian Americans and Pacific Islanders. Dig Liver Dis 2007;39:549-56.
16. Lee JG, Lee S, Kim YJ, Cho BM, Park JS, Kim HH, *et al.* Association of chronic viral hepatitis B with insulin resistance. World J Gastroenterol 2012;18:6120-6.
17. Sangiorgio L, Attardo T, Gangemi R, Rubino C, Barone M, Lunetta M. Increased frequency of HCV and HBV infection in type 2 diabetic patients. Diabetes Res Clin Pract 2000;48:147-51.
18. Kremsdorf D, Soussan P, Paterlini-Brechot P, Brechot C. Hepatitis B virus-related hepatocellular carcinoma: Paradigms for viral-related human carcinogenesis. Oncogene 2006;25:3823-33.
19. Irshad M, Irshad K, Nag P. Insulin resistance in liver diseases. Macedonian J Med Sci 2011;4:201-9.
20. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. Science 1993;259:87-91.
21. Serino M, Menghini R, Fiorentino L, Amoruso R, Mauriello A, Lauro D, *et al.* Mice heterozygous for tumor necrosis factor-alpha converting enzyme are protected from obesity-induced insulin resistance and diabetes. Diabetes 2007;56:2541-6.
22. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 1972;97:142-5.
23. Reinhold JG. Biuret method. In: Reiner M, editor. Standard Method of Clinical Chemistry. 1st ed., Vol. 1. New York: Academic Press; 1953. p. 88.
24. Spencer K, Price CP. Influence of reagent quality and reaction conditions on the determination of serum albumin by the bromcresol green dye-binding method. Ann Clin Biochem 1977;14:105-15.
25. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic

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pyruvic transaminases. Am J Clin Pathol 1957;28:56-63.
26. Klein B, Read PA, Babson AL. Rapid method for the quantitative determination of serum alkaline phosphatase. Clin Chem 1960;6:269-75.
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
28. Mohammad Alizadeh AH, Fallahian F, Alavian SM, Ranjbar M, Hedayati M, Rahimi F, *et al.* Insulin resistance in chronic hepatitis B and C. Indian J Gastroenterol 2006;25:286-9.
29. Kim KH, Shin HJ, Kim K, Choi HM, Rhee SH, Moon HB, *et al.* Hepatitis B virus X protein induces hepatic steatosis via transcriptional activation of SREBP1 and PPARgamma. Gastroenterology 2007;132:1955-67.
30. Kim K, Kim KH, Cheong J. Hepatitis B virus X protein impairs hepatic insulin signaling through degradation of IRS1 and induction of SOCS3. PLoS One 2010;5:e8649.
31. Dai FH, Zeng WQ, Jiang CY. Assessment of the factors associated with insulin resistance in patients with chronic hepatitis B infection. Zhonghua Gan Zang Bing Za Zhi 2012;20:517-21.
32. Wang CC, Hsu CS, Liu CJ, Kao JH, Chen DS. Association of chronic hepatitis B virus infection with insulin resistance and hepatic steatosis. J Gastroenterol Hepatol 2008;23:779-82.
33. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev 2005;26:19-39.
34. Hajjou M, Norel R, Carver R, Marion P, Cullen J, Rogler LE, *et al.* cDNA microarray analysis of HBV transgenic mouse liver identifies genes in lipid biosynthetic and growth control pathways affected by HBV. J Med Virol 2005;77:57-65.
35. Kim K, Kim KH, Kim HH, Cheong J. Hepatitis B virus X protein induces lipogenic transcription factor SREBP1 and fatty acid synthase through the activation of nuclear receptor LXRalpha. Biochem J 2008;416:219-30.
36. Steinberg GR, Michell BJ, van Denderen BJ, Watt MJ, Carey AL, Fam BC, *et al.* Tumor necrosis factor alpha-induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. Cell Metab 2006;4:465-74.
37. Cawthorn WP, Sethi JK. TNF-alpha and adipocyte biology. FEBS Lett 2008;582:117-31.