Review Article

Toll-like Receptor-8, Liver Enzymes and some Antioxidant Markers among Hepatitis C Virus infected Subjects in Kano Metropolis

*Isah Suleiman Yahaya[1,4,6], Istifanus Solomon[1], Munir Jabir[2], Hamidu Labaran[1], Kabir Magaji Hamid[5], Lawal Dahiru Rogo[1], Ahmad Muhammad Bello[1], Muhammad Yalwa Gwarzo [1,6], Danladi Suleiman Bala[1], Zakariya Abdulganiy [1], Fatima Bashir Shema[1], Nasiru Magaji Sadiq[4], Mustapha Ahmed Yusuf[3,4], Aliyu Aminu[3,4], Umar Usman Yahya[4], Muhammad Habiba Yahaya[6], Auwal Idris Kabuga[3,4], Muhammad Akram[7]and Yusuf Aminu[8]

[1]Department of Medical Laboratory Science, Bayero University, Kano, Nigeria, [2]University Health Services Department, Federal University of Technology, Babura, Jigawa state, Nigeria, [3]Department of Microbiology and Parasitology, Bayero University, Kano, Nigeria, [4]Department of Medical Microbiology, Aminu Kano Teaching Hospital, Kano, Nigeria, [5]Department of Immunology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria, [6]Chemical Pathology Department, Aminu Kano Teaching Hospitaly, Kano, Nigeria, [7]Department of Eastern Medicine, Government College University Faisalabad-Pakistan, [8]Department of Medical Laboratory Science, Kaduna State University, Nigeria

Abstract

Background: Hepatitis C virus (HCV) remains a major global health issue, contributing to significant hepatic damage. The quest to avert morbidity and mortality-related complications requires concerted efforts of researchers through exploring Toll-like receptor-8 (TLR8), superoxide dismutase (SOD), and glutathione peroxidase (GPx) biomarkers**.** The aim of the study was to assess TLR8, some liver enzymes, and some antioxidant markers among subjects with HCV infection in Kano metropolis. **Methodology:** One hundred and twenty subjects comprising of 80 HCV cases and 40 healthy individuals as controls participated in this study. Serum alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Gamma glutamyl Transferase (GGT) were determined using enzymatic kinetic methods, while serum TLR8, SOD and GPx were measured using sandwich ELISA technique and body mass index (BMI) was calculated using the weight and height of the subjects by standard method. **Results:** The mean ALT and TLR8 were significantly higher, while SOD and GPx were significantly lower in HCV subjects compared with controls. BMI, AST, and ALP were not significant in HCV subjects compared with controls. There was a significant positive correlation between serum TLR8 and ALT (r=0.56, p=0.02), a significant negative correlation was observed between serum TLR8 and SOD (r=0.50, p=0.03). There was no significant correlation between TLR8 with AST, ALP, GGT and GPx (p > 0.05). **Conclusion:** These findings suggest that increased TLR8 activity may be associated with hepatic inflammation and reduced antioxidant defense in HCV patients, highlighting the potential role of TLR8 and antioxidant marker in HCV pathogenesis.

Key words: Antioxidant, Biomarker, Immunochemistry, Liver Enzymes, Toll like receptor

Introduction

Background

According to the World Health Organization (WHO), 3–4 million individuals contract hepatitis C virus (HCV) yearly, and about 130–170 million people have chronic infection associated with liver inflammation. Illnesses associated with HCV caused over 350000 deaths annually, HCV infection predisposed one to some cancers such as hepatocellular carcinoma (HCC) and lymphomas in humans making it's a major global health concerns[1]. It is a positive-sense, encapsulated, single-stranded RNA (ssRNA) virus that belongs to the *Flaviviridae* family and is between (55–65 nm) in size [2]. The HCV infection are different in genotypes strains at 30%–35% of nucleotide sites of the virus, subtype-specific strains differ at less than

Address for correspondence: Dr. Isah, Suleiman Yahaya, Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Bayero University Kano, Nigeria. Telephone: +2348035165618. Email: isyahaya.mls@buk.edu.ng ; ORCID:0000-0003-2313-6915

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15% of nucleotide sites of the virus [3], with extremely little chance of sexual or vertical transmission [4]. It is a blood-borne infection and the main populations at risk due to this route of transmission include those who use intravenous drugs (IDUs), receive blood products, and occasionally those undergoing hemodialysis. Intra-hospital (nosocomial) transmission is another common route of HCV transmission, which occurs when sterilisation and hygiene procedures are not adequately followed in the clinic [5]. Circumcision, genital mutilation, ceremonial scarification, traditional tattooing, and acupuncture are among the cultural or ritual practices that have been suggested as the possible historical route of HCV transmission [6].

When damage or pathogen invasion occurs, the innate immune system offers a prompt protective mechanism that enables the adaptive immune system to launch an antigen-specific response [7]. The core of the innate immune response is the family of pattern recognition receptors (PRRs) known as toll-like receptors (TLRs) [8]. Toll-like receptor-8 (TLR8) is a protein that in humans is encoded by the TLR8 gene and it is designated as cluster of differentiation 288 (CD288) [9]. It can recognize GU-rich oligonucleotidesis an endosomal receptor that recognizes single-stranded RNA (ssRNA) viruses such as Influenza, Sendai, and Coxsackie B viruses among others [10]. TLR8 binding to the viral RNA recruits MyD88 and leads to activation of the transcription factor NF-κB and an antiviral response [11].

The body has proteins called enzymes that reduce activation energy to speed up specific chemical reactions [12]. Liver enzymes such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), and gamma-glutamyl transferase (GGT) are enzymes that are mainly made in the liver. If the liver is injured, AST, ALP, GGT and ALT pass into the bloodstream making the values of the liver enzymes to be higher than normal that indicates liver damaged [13].

An antioxidant is a molecule that can slow or even stop the oxidation of other molecules. It can also be an ion or a relatively stable radical that is employed as an indicator of oxidative stress (like albumin) [14-15]. Biomarkers of oxidative stress can be classified as molecules that are altered by interactions with reactive oxygen species (ROS) in the microenvironment and molecules of the antioxidant system that change in response to elevated redox stress [16-17]. Reactive oxygen species are produced as metabolic by-products of regular cellular activity. Catalase, glutathione peroxidase (GPx) and superoxide dismutase (SOD) are the enzymes that shield cells from the harmful effects of ROS [18-19]. All living cells contain the enzyme SOD and GPx. An enzyme is a substance that quickens specific bodily chemical processes. In cells, SOD aids in the breakdown of potentially hazardous oxygen molecules, tissue injury might be avoided in this process and GPx in turn aids in maintaining intracellular homeostasis, redox balance, and the prevention of lipid peroxidation [20]. Assessment of TLR8 and some antioxidant markers among HCV infected subjects in Kano being a low-resource setting and how it could benefit the society was the main focus of this study.

Methodology

The study was a case-control study conducted in Murtala Muhammad Specialist Hospital (MMSH) and Muhammad Abdullahi Wase Specialist Hospital (MAWSH) and was an exploratory or baseline study before commencing treatment. This comprised eighty (80) HCV subjects, confirmed by laboratory diagnosis to have HCV by the Gold rapid screen test strip by micropoint with pool positive and negative samples as quality controls and forty (40) apparently healthy volunteers served as controls with participant's age range between 18-70 years. The BMI was determined using standard technique as described by Isah et al. [21] and Isah et al. [22]. Serum levels of AST and ALT were determined using enzymatic the kinetic methods as described by Huang et al. [23]. Serum ALP was determined using kinatic method according to the technique described by Isah et al. [24]. Serum GGT was determined using kinetic method according to the technique described by Angeli et al. [25] and Li et al. [26] and Toll-like receptors-TLR8, SOD and GPx were measured by the Sandwich ELISA

technique using reagents supplied by Melsin Medical Co., LTD, Kuancheng District, Changchun, Jilin Province, China.

Inclusion criteria

This includes subjects with HCV within the ages of 18 – 70 years who consented to participate in the study. Subjects with HCV who have no any underlying diseases like TB, HIV and HBV among other viral infections and have not commenced treatment (naïve patients). Control subjects were HCV negative and consented to participate in the study.

Exclusion Criteria

These were subjects co-infected with HCV and other infectious agents like TB, HIV or HBV. Also, subjects with HCV who have commenced treatment and pregnant female participants were also excluded.

Ethical Consideration

The Kano State Ministry of Health's Ethical Committee approved this study, with reference number NHREC/17/03/2018, dated April 19, 2021. Before any BMI and sample collection were performed, participants gave their consent and were satisfactorily informed about the goal and methodology. The study complied with the Helsinki Declaration's requirements.

Sample Collection and Laboratory Methods

Five (5ml) of blood was collected via venipuncture from all participants into a plain vacutainer. The serum collected was stored in cryotubes at -20^{o}C until used [21] for TLR8, ALT, AST, ALP, GGT, SOD and GPx assays.

Data analysis

Data was analyzed using Statistical Package for Social Sciences version 21.0 (SPSS) statistical software. Normality testing was performed and the data were normally distributed. The mean and standard deviation were computed and results were expressed as mean±SD. The student t-test was used to compare differences between means of two groups. Correlation was performed using Pearson's Correlation Coefficient. Statistical significance was set at $p<0.05$.

Results

The results obtained from the present study are presented in Tables I-IV, respectively. Table I describes the distribution of patients according to age and gender. The highest frequency was observed in the age range between 18-30 years, with a percentage of 64.7% in male subject, while in female subject, the highest frequency was observed in the age range between 31-40 years, with a percentage of 100% and the lowest frequency was observed in the age group >60 years old in male subject, with a percentage of 0%. In total, males had a higher frequency 68(85%) of hepatitis C virus disease than females, who had a frequency of 12 (15%).

Table 1: Distribution of subjects According to Age and Gender

Age group (yrs)	M(n, %)	F(n,%)	*p-value*
18-30	44(64.7%)	0(0%)	0.35
31-40	20(29.4%)	12(100%)	
41-50	4(17.7%)	0(0%)	
>60	0(0%)	0(0%)	
Total	68(85%)	12(15%)	

*M=male, F=female, yrs= years, *Chi square test, n = frequency, % = percentage, p value < 0.05 at 95% confidence interval.*

Table II shows the body mass index mean value in HCV subjects and controls. The mean± SD of BMI in subjects (23.40 ± 2.85 kg/m^2) was not statistically significant (p = 0.14) when compared with the controls (21.9 ± 3.40 kg/m^2) respectively.

Table 2: Body Mass Index (mean±SD) in HCV subjects and controls

Variables	Subjects (n=80)	Controls (n=40)	*t-value*	*p-value*
Height (m)	1.73 ± 0.08	1.74 ± 0.08	-0.14	0.88
Weight (kg)	70.05 ± 8.91	66.05 ± 8.63	1.44	0.15
BMI(kg/m^2)	23.40 ± 2.85	21.9 ± 3.40	1.50	0.14

*p≤ 0.05 (significant of Independent t-test) for subject Vs Control for Analysis *; n=Number of Subject; BMI= Body Mass Index; HCV= Hepatitis C Virus; SD= Standard deviation.*

Table III demonstrates the toll-like receptor 8 liver and oxidative enzymes (mean±SD) in HCV subjects and controls. The mean ± SD of ALT (39.30±15.65 U/L), TLR8 (6.33 ± 3.16 ng/ml) of subjects were significantly (p = 0.00) higher compared with controls (19.95±7.30 U/L, 3.05±0.97 ng/mL) respectively. The mean± SD of SOD (16.18± 3.48ng/mL) and GPx (2.66± 0.51 ng/mL) of subjects were significantly (p = 0.00) lower compared with controls (21.05± 5.80 ng/mL and 4.21± 0.54 ng/mL) respectively. However, there is no statistically significant difference (p>0.05) in ALP, AST and GGT in HCV subjects compared with the control groups.

Table 3: Toll-like receptor 8, Liver and oxidative Enzymes (Mean±SD) in HCV subjects and controls

Variables	Subjects (n=80)	Controls (n=40)	*t-value*	*p-value*
ALT (U/L)	39.30±15.65	19.95±7.30	5.01	0.00*
ALP (U/L)	174.55 ± 56.44	169.60 ± 26.84	1.43	0.23
AST (U/L)	78.91 ± 39.42	32.00 ± 14.54	1.74	0.09
GGT(U/L)	11.50 ± 9.30	9.21 ± 6.24	1.31	0.19
TLR8(ng/mL)	6.33 ± 3.16	3.05±0.97	2.65	0.00*
SOD (ng/mL)	16.18± 3.48	21.05± 5.80	-2.26	0.00*
GPx (ng/mL)	2.66± 0.51	4.21± 0.54	-3.14	0.00*

*p≤ 0.05 (significant of Independent t-test) for subject Vs Control for Analysis *; n=Number of Subject; AST= Aspartate transaminase; ALT = Alanine transaminase; ALP= Alkaline phosphatase; GGT= Gamma-glutamyl transferase; TLR8 = Toll-like receptor-8; SOD = superoxide dismutase; GPx = glutathioneperoxidase; HCV = Hepatitis C virus, HCV= Hepatitis C Virus; SD= Standard deviation.*

Table IV depicts the correlation between toll-like receptor 8 with liver and antioxidant enzyme parameters among HCV subjects. There was a positive correlation between TLR8 & ALT (r=0.56, p=0.02) and a negative correlation between TLR8 & SOD (r=-0.50, p=0.03). No significant correlation (p >0.05) was observed between TLR8 with AST, ALP, GGT and GPx.

Table 4: Correlation between toll like receptor 8 with liver and antioxidant enzymes parameter among HCV subjects

Variables	*r - value*	*p-value*
TLR8 & ALT	0.560	0.023*
TLR8 & AST	0.287	0.221
TLR8 & ALP	0.003	0.989
TLR8 & GGT	0.091	0.702
TLR8 & SOD	-0.495	0.030*
TLR8 & GPx	-0.208	0.121

*#=determined by pearsons correlation; *p= Correlation is significant at ≤ 0.05 levels (2-tailed); CI=95% Confidence Interval; r = strength of correlation; n=Number of Subject; AST= Aspartate transaminase; ALT = Alanine transaminase; ALP= Alkaline phosphatase; GGT= Gamma-glutamyl transferase; TLR8 = Toll-like receptor-8; SOD = superoxide dismutase; GPx = glutathioneperoxidase; HCV = Hepatitis C virus, HCV= Hepatitis C Virus*

Discussion

Hepatitis C virus causes severe liver damage, including cirrhosis, liver failure, and hepatocellular carcinoma (HCC) [27]. The global, regional and national burden of morbidity and mortality associated with HCV infection, based on prevalence, incidence and transmission is greatly alarming [28]. Therefore, the need for reduction of global mortality and morbidity related to chronic hepatitis C is becoming greatly important [29]. Numerous biochemical parameters are useful for assessing the integrity and severity of hepatitis C infection [30]. However, few immunological assay variables exist, especially innate immune and antioxidant markers essential for effective responses.

In the present study, high prevalence of Hepatitis C virus diseases was observed in age 18-30 years and the lowest was observed in the age >60 years. Our report is similar to the work of Jemilohun et al. [31], but at variance with the report of Umumararungu et al. [32]. El-Adly et al. [33] also disagreed with our finding. Ugwu et al. [34] reported that the majority of donors are between the ages of 18 and 27. Young adult males are more affected by HCV due to increased engagement in high-risk behaviors and

greater exposure to unsafe medical and social practices [31]. However, risk factors for HCV transmission were excluded from this study in order to avoid overestimating the issue and this study acknowledge lack of PCR confirmation to confirmed the HCV positive subject particularly in a low economic setting.

Our finding reveals that males had a higher frequency of HCV infection than females. This is in agreement with the findings of Umumararungu et al. [32], Muttaka et al. [35] and Abdel-Gawad et al. [36]. This finding is in disagreement with the findings of Esmaeili et al. [37], Ndako et al. [38], as they reported that females were more infected than males. The lifestyle of males, such as the *use of contaminated blood products, injection drugs and others,* might make them more exposed to various risk factors [36]. The predominance of male donors in the study may explain the finding and potentially limit the inferential validity of conclusions about gender differences in HCV infection.

In the present study, the mean values of BMI in HCV subjects were not statistically significant in the subjects group when compared with the control group. This is similar to the report of Corey et al. [39], but El Kassas et al. [40] disagreed with our finding. HCV subjects in steady state show near-normal BMI due to slow viral catabolism and youthful immune response [41].

In the current investigation, the mean value of serum ALT was statistically significantly higher in the subject group than the control group. Our report is in conformity with the findings of Akkaya et al. [42], Hajarizadeh et al. [43] and Marei et al. [44]. Gradual hepatocyte damage causes aminotransferases to leak into blood, may produce elevated circulating enzyme levels systemically [45].

The finding of this study reveals that the mean values of AST, GGT and ALP were statistically not significant in the subject group compared to control group. Our report is in agreement with the report of Marei et al. [44]. ALT is highly concentrated in the liver, while AST exists in multiple organs including heart, muscle, kidney, brain, pancreas, and lung. ALP is widespread, mainly in bone and liver, and GGT is abundant in liver, kidney, pancreas, and intestine. [13]

Antioxidant enzymes protect cells by reducing H_2O_2 and lipid peroxides, preventing reactive oxygen species formation, and minimizing oxidative tissue damage, thereby maintaining cellular integrity and reducing harmful oxygen molecule effects. [46] Our recent research indicates that the mean values of serum SOD and GPx were statistically significantly lower in the subject group compared to the control group. Our finding is in conformity with the reports of Ivanov et al. [47] and Paracha et al. [48]. However, Osman et al. [49] and Ismail et al. [50] reported otherwise. Numerous RNA viruses, like the HCV virus that reproduces in the cytoplasm, share a common mechanism in which they modify the cellular environment through the dysregulation of antioxidant and oxidative stress mechanisms [51]. Reduced antioxidant markers increase oxidative stress in infected cells, causing hepatic damage worsened by immunosuppression and viral exposure [52]. Antioxidants hinder HCV replication, improve liver enzymes, protect cells, and enhance interferon effectiveness against infection [53].

The innate immune system is essential for early pathogen detection, controlling inflammation, activating adaptive immunity, and combating viral infections using pathogen-associated molecular patterns (PAMPs), pattern recognition receptors (PRRs), and toll-like receptors (TLRs) to recognize and respond to invasive pathogens effectively [54-55]. The current study reveals that the mean value of serum TLR-8 was statistically significantly higher in the subjects group than the control group. This is in agreement with the findings of Firdaus et al. [56], Wang et al. [7] and Kayesh et al. [55]. HCV PAMPs trigger innate and adaptive immunity through PRRs recognizing them as non-self. TLR8, an endosomal ssRNA receptor, interacts with HCV components, inducing antiviral responses via TNF-α and IFN-β in monocytes and dendritic cells, promoting inflammation and viral inhibition [55-57].

In this finding, a statistically significant positive correlation was observed between TLR8 & ALT. However, no statistically significant correlation was observed between TLR8 with ALP, AST and GGT. This is in conformity with the findings of Kayesh et al. [55], where they reported that an increase in TLR8 in HCV may lead to an increase in ALT. TLR8, a component of the liver immune system that is present in both liver parenchymal cells and other immune cells, plays a crucial role in innate immune mediation and the induction of acquired immunity, hence enhancing the immune response's overall effectiveness [58].

TLR8 plays a key role in HCV, triggering antiviral and inflammatory pathways [58]. Our findings reveal a statistically significant negative correlation between TLR8 and SOD. However, no statistically significant correlation was observed between TLR8 and GPx. These results could suggest that in HCV patients, elevated TLR8 might result in decreased SOD levels. Increases in TLR8 have been documented to increase viral infection defenses [59]. Antioxidant markers are essential enzymes converting superoxide to hydrogen peroxide, defending cells against reactive oxygen species–induced oxidative damage [20]. HCV subjects may experience oxidative stress due to decreased SOD increasing ROS [47]. Decreased antioxidant markers may indicate HCV immune evasion and oxidative stress–mediated hepatic damage, though observed correlations remain unadjusted and exploratory.

Conclusion and Recommendation

The study shows elevated TLR8 and ALT with normal other liver enzymes, alongside reduced SOD and GPx, and significant associations between TLR8, SOD, and ALT in stable HCV subjects. As baseline research, larger samples, Western blot analysis, and cohort studies are recommended. In low-income and middle-income settings, routine liver enzyme monitoring occurs, but adding TLR8 and antioxidants could enhance HCV management locally.

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Conflict of Interests
The authors affirm that there is no conflict of interest associated with this paper's publication.

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Author's contributions
All author contributed equally to the completion and approval of this work, and they are all accountable for its integrity and correctness.

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