

Prevalence of Hepatitis B Virus (Hbv) and Hepatitis C Virus (Hcv) and their effects on serum albumin and liver aminotransferases in pregnant women in Jos

Onwuiiri FC^{1*}, Ndako JA² and Onwuliri EA³

¹Professor, Department of Plant Science and Technology, University of Jos, Plateau State Nigeria

²Senior Lecturer, Department of Biological Sciences, Landmark University Omuaran Kwara State, Nigeria

³Senior Lecturer, Department of Pharmaceutics and Pharmaceutical Technology, University of Jos, Plateau State, Nigeria

Abstract

Infections due to Hepatitis B and Hepatitis C viruses are significant health problems around the globe, Nigeria inclusive. Asymptomatic Hepatitis B and C infections are common and when occurring in pregnancy can be transmitted to the new born. To determine the prevalence rate of asymptomatic hepatitis B and C infections among pregnant women, 406 pregnant women attending antenatal clinic at University Health centre and Our Lady of Apostle hospital, all in Jos, Plateau State were recruited for the study. The study was also carried out to determine whether liver aminotransferases and serum albumin can be affected by hepatitis infection during pregnancy. Demographic and past clinical histories were obtained using a questionnaire. Serum samples from each study subject were tested using third-generation enzyme immunoassay kits for hepatitis B surface antigen (HBsAg) and antibodies against hepatitis C (HCV). Serum Alanine Aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were also estimated in all subjects using Reitman-Frankel method. Also serum albumin was measured in all subjects using Bromocresol green (BCG) method by Teitz. The results showed that 10.0% and 1.2% of the 406 blood samples tested positive on HBV and HCV, respectively. Furthermore, 1.2% of the 406 blood samples tested positive with both HBV and HCV. The mean AST levels for HBsAg negative and positive subjects were 10.55 ± 0.05 and 12.16 ± 0.29 , respectively while the mean ALT levels were 5.54 ± 0.005 and 8.01 ± 0.01 , respectively. The mean AST for anti-HCV negative and positive subject were 10.67 ± 0.01 and 9.01 ± 0.05 , respectively while ALT were 5.71 ± 0.01 and 4.01 ± 0.05 , respectively. There was a significant increase in levels of AST and ALT between the HBsAg positive and negative pregnant subjects ($P < 0.05$). Furthermore, the mean serum albumin level for HBsAg positive and negative pregnant subjects were 30.60 ± 2.75 and 35.58 ± 3.82 , respectively. Also, the mean albumin level for HCV positive and negative pregnant subjects were 28.5 ± 2.12 and 35.55 ± 3.7 , respectively. There was a significant increase in albumin level between HBsAg and HCV positive and negative pregnant subjects ($P < 0.05$). HBV and HCV infection can be present in pregnant women and can alter liver aminotransferases and serum albumin. Routine screening of pregnant women for HBV and HCV should be instituted in order to detect infection early and prevent or reduce vertical or prenatal transmission.

Introduction

Viral hepatitis is the inflammation of the liver caused by infection with hepatitis viruses. Infection with hepatitis B virus or hepatitis C virus are public health problems and are highly endemic in Sub-Saharan Africa [1,2]. Worldwide, there are about 350 million HBV carriers [3]. HBV and HCV infections are major causes of morbidity and mortality.

Hepatitis B virus is a DNA virus of the family hepadnaviridae and the causative agent of hepatitis B infection [4]. It is 50-100 times more infectious than HIV and 10 times more infectious than hepatitis C. Many carriers do not realize they are infected with the virus, thus it is referred to as a 'silent killer' [5].

Hepatitis C virus (HCV) is an RNA virus of the flaviviridae family and appears to have humans and chimpanzees as the species susceptible to its infection [6]. About 170 million people are infected with HCV worldwide [7].

Both viruses are transmitted through contact with infected blood, sexual intercourse and vertical transmission (mother-to-child) [8]. Maternal mortality has been shown to increase in pregnant women with liver cirrhosis.

HBV and HCV account for a substantial portion of liver diseases worldwide and infected individuals can remain asymptomatic for decades. However, more than 80% of them become chronic carriers which result in an increased risk of liver cirrhosis, liver cancer and liver failure 20-30 years later. They share similar modes of transmission. Co-infection is not uncommon especially in areas of high prevalence and among people at high risk for parental infection.

In Nigeria, the prevalence rate of HBV and HCV in pregnant women differ from one locality to another. Yakasai *et al.* [9] reported a prevalence of 7.9% HBsAg in pregnant women in Kano, Nigeria. While Oladeinde *et al.* [10] reported a prevalent rate of 8 (2.2%) and 3 (0.8%) of HBV and HCV infections, respectively among pregnant women in Benin city, Nigeria. Co-infection of HBV and HCV seems to result in more severe disease than either infection alone.

Globally hepatitis B virus (HBV) infection is the most common form of chronic hepatitis around the world. Chronic carriers can

Correspondence to: Onwuiiri FC, Department of Plant Science and Technology, Applied Microbiology Unit, University of Jos, Nigeria, Tel: +2348065302804; E-mail: faconwuliri@yahoo.com

Received: March 22, 2017; **Accepted:** April 19, 2017; **Published:** April 22, 2017

continue to transmit the disease for many years, before becoming symptomatic [11]. Together hepatitis B and C represent one of the major threats to global health. Infection occurs very often in early childhood when it is asymptomatic and then lead to the chronic carrier state; chronic HBV infection leads to increase risk for chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma (HCC) [11].

In many developed countries (e.g. those in Western Europe and North America) patterns of transmission are different from those in developing countries. The majority of infections in developing countries are transmitted by sexual activity and drug use especially among young adults [12].

Hepatitis B is a major infectious occupational hazard of health worker. Hepatitis B Virus is not spread by contaminated food or water and cannot be spread casually in the work place. Hepatitis B is transmitted through contact with an infected person's blood, semen or other body fluid.

Materials and methods

Study area

This study was carried out in the University Health Centre (UHC) and Our Lady of Apostle (OLA) hospital, both located in Jos North Local government of Plateau State.

University Health Centre is located in the permanent site of University of Jos, and is positioned to take care of the health needs of students, staff and their dependents. Anti-natal care is also given to both staff and students. OLA hospital is located in the heart of the city of Jos being a popular maternity place for different ethnic and religious groups, both urban and rural women use OLA hospital for their ante-natal care center. the study was conducted on 406 pregnant women attending ante-natal clinic in UHC and OLA hospital

Ethical clearance

Ethical clearance were sought and obtained from UHC and OLA hospital management before commencing the study. The nature of the study was explained to the patients in a simple language to their understanding and their consent was obtained before enrolment into the study

A well designed personal data information sheet was used to gather information regarding age, surgery, HBsAg vaccination, blood transfusion. Blood sample was obtained by venipuncture. Ten millilitre of blood sample was collected from a prominent vein on the arm of each of the women into z_{10} tube. It was allowed to clot and then centrifuged at 1000rpm for 5 minutes and serum separated and stored at -20°C .

The samples were evaluated for the presence of HBV surface antigen and anti-HCV using a rapid lateral chromatographic immunoassay kit (WHOBC-ACON, Biotech, Hangzhou, China) and a rapid visual immunoassay kit (WHOBC – Lebman, Hamburg, Germany). Assays were done at room temperature; the pouch was opened under room temperature because the test strips need to equilibrate with the room temperature prior to testing. The serum was also brought out of refrigerator, for it also to equilibrate with room temperature before testing.

Test procedure

With the arrows pointing downwards, it was immersed into the serum for 10-15 seconds. The maximum line (max) on the strip was observed in order to avoid exceeding the line. The strip was then placed on a non-absorbent surface. The timer was then set for fifteen (15)

minutes, awaiting the red line to appear or not. Two distinct red lines, one on the control region (C) while the other on the test (T) region.

Interpretation of result

Negative: Only one color band appears on the control region. No apparent band on the test region. This indicates that there is no detectable anti – HCV in the whole blood.

Positive: Distinct color bands appears on the control and test region. Both test line and control line indicate that the specimen contains detectable amount of anti-HCV.

Invalid: No visible band at all or only one colored band appears on the test region, this is an indication of a possible error in performing the test.

Liver function test

Liver Function tests (LFTs) are groups of clinical biochemistry laboratory assays designed to give information about the state of a patients Liver.

The parameters measured include Prothrombin time (PT), albumin, bilirubin (direct and indirect). Liver transaminases AST/ALT. These are called Liver damage test-biomarker of Liver injury in a patient with some degree of intact liver function. For the purpose of this research, albumin, Aspartate transaminase (AST), Alanine transaminase (ALT) were determined on the pregnant women who are positive to HBV and HCV infection.

Albumin test

Albumin is a protein made specifically by the liver. It is the main constituent of total protein (the remaining from globulins). Albumin levels are decreased in chronic liver disease such as cirrhosis (Medline plus Encyclopedia).

Principle: The measurement of Serum albumin is based on its quantitative binding to the indicator 3,3:5,5 – tetrabromom-cresol sulphonephthalein (bromocresol green, BCG). The albumin – BCG – complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample.

Statistical analysis

The prevalence of each viral infection (HBV and HCV) was determined from the proportion of the positive individuals in the total population under consideration and expressed as a percentage. The chi-square test was employed to determine the relationships between age and presence of risk factors with HBV and HCV infection at $P < 0.05$.

Also t-test for AST, ALT and albumin level among the pregnant women were tested to determine if their level is significant or not in the study at $P < 0.05$.

The results of the prevalence study are presented in the Table 1. It shows that out of 406 pregnant women tested, 41(10%) were positive for HBsAg and 5 (1.2%) were positive for anti-HCV giving an overall prevalence of 10% and 1.2%, respectively. Also the study shows that out of 406 pregnant women tested, 5 (1.2%) were positive for HBV and HCV infection showing a prevalence of 1.2% of co-infection with HBV and HCV. The study shows that the prevalence of HBsAg and HCV is significant at $P < 0.05$.

Table 2 shows prevalence of HBsAg and anti-HCV among the pregnant women based on age group. The prevalence of HBsAg was

Table 1. Prevalence of HBV and HCV in Pregnant Women

N = 406				
Hepatitis Serology	No. Positive Result (%)	No. Negative Result (%)	X ²	P-value
HBsAg	41 (10.0)	365 (90)	258.562	0.000
Anti HCV	5 (1.2)	401 (98.7)	389.244	0.000
HBV/anti HCV	5 (1.2)	401 (98.7)	389.244	0.000

Table 2. Prevalence of HBV and Anti HCV among pregnant women based on Age group.

Age group (Yrs)	Number tested	No HBsAg positive (%)	No of HCV positive (%)	X ²	P-value
15-24	162	24 (15.0)	0 (0.0)	24.00	0.000
25-34	114	11 (9.5)	5 (4.8)	52.25	0.134
35-44	81	6 (6.6)	0 (0.0)	6.000	0.014
45-54	49	0 (0.0)	0 (0.0)	0.000	0.000

highest among the age group (15-24years) while Anti-HCV was seen in age group 25-34years. The prevalence in age group 15-24 year was significant at (P < 0.05) while the prevalence rate in age group of 25-34 year and 35-44 year were not significant when compared at (P < 0.05).

Table 3 shows the percentage of those with history of blood transfusion that were positive for HBsAg and anti HCV as 4.2% and 0.8% respectively, and those with no history of blood transfusion that were positive for HBsAg and anti HCV as 33.3% and 3.3% respectively. There was no significant difference when those who had blood transfusion and those who did not were compared. Also there was no significance difference in HCV individuals. The percentage of those with history of surgery that were positive for both HBcAg and anti HCV as 33.3% and 3.3% respectively. There was no significant difference when those who had blood transfusion and those who did not were compared. Also, there was no significance difference in HCV individuals. The percentage of those with history of surgery that were positive for both HBsAg and Anti HCV were 6.9% and 0%, respectively and those with no history of surgery who were positive for HBsAg and anti HCV were 14.2% and 0%, respectively. However, there was no significant difference between them. Furthermore, the percentage of those with family history of hepatitis who were positive for HBsAg and anti HCV were 9.5% and 1%, respectively and those who were not positive for HBsAg and Anti HCV were 10.9% and 1.8%, respectively. There was also no significant difference when compared.

Table 4 shows the mean AST levels for HBsAg negative and positive subjects were 10.55 ± 0.05 and 12.16 ± 0.29, respectively while the mean ALT levels were 5.54 ± 0.005 and 8.01 ± 0.01, respectively. There was a significant difference in mean AST level between those who were HBsAg positive and those that were negative. Also there was a significant difference in their mean ALT levels (P < 0.05) when compared. The mean AST level for anti-HCV negative and positive subjects were 10.01 ± 0.09 and 10.67 ± 0.01 respectively while their mean ALT were 9.01 ± 0.05 and 5.21 ± 0.01. There was a significant difference in mean AST and ALT levels between those who were HCV positive and those who were negative when compared.

Table 5 shows that the mean albumin level for HBsAg negative and positive subjects were 35.58 ± 3.82 and 30.60 ± 2.75, respectively. There was a significant difference when compared. Also the mean albumin level for anti HCV negative and positive subjects were 35.55 ± 3.7 and 28.5 ± 2.12. There was a significant difference when compared at P < 0.05.

Discussion

The prevalence of hepatitis B and C infection varies in different parts of the world from country to country, and from one region to

another region and from one population group to another in a country [13] and since pregnant women have depressed immunity, infections of HBV and HCV are of clinical importance.

The study shows that the prevalence (10.0%) of HBV in the pregnant women is within the intermediate endemicity according to WHO criteria [14]. Uneke *et al.* [15] classified high endemicity from HBV infection and defined it as HBsAg greater than 7% in an adult population. This also supports the WHO (1990) report for Nigeria as highly endemic area with prevalence greater than 8%. The prevalence of HBsAg found in pregnant women attending antenatal in Jos falls within the range of reports given in other studies carried out in other parts of Nigeria, Africa and the rest of the world. In reported studies for hepatitis B carried out in some parts of Nigeria, there were higher prevalence rates of 12.8% in Minna [16], 15.8% in Maiduguri [17], 11% in Makurdi [18]. Lower prevalence's reported include 2.19% in Benin City [19], 8.3% in Zaria [20] and 5.7% in Ilorin. In some African countries, there were high prevalence rates of 17.3% in Burkina faso. Lower reports were 5.3% in Ethiopia, 6.3% in Tanzania. In comparison to other findings from the rest of the world were 2.11% in Northern Turkey 12% in Taiwan. These variations noticed may be related to the peculiarities in the modes of transmission of HBsAg and HCV dictated by socio cultural practices and environmental factors.

A prevalence of 1.3% HCV in the studied population was found to be low when compared to other studies carried out in Nigeria. Paul *et al.* reported a 12% in South West Nigeria and 2.5% in Maiduguri [17]. This variation noticed may be related to the peculiarities in the modes of transmission of HCV dictated by socio-cultural and environmental factors.

My findings compared to findings from other African countries, showed that there were higher rates of 17-26% in Egypt [21], 2.6% Cote

Table 3. Prevalence of HBV and Anti HCV in relation to some associated risk factors among the pregnant women. Significance = P < 0.05, X² = Chi-square, HBT = History of blood transfusion, HS = History of surgery, FHHI = Family history of hepatitis infection.

Risk Factors	Number Tested	No of HBsAg positivity (%)	No of Anti HCV positivity (%)	X ²	P-value
HBT	Yes	325	13 (4.2)	HBV 1.67 HCV 1.0	0.197 0.317
	No	81	28 (33.3)		
HS	Yes	235	16 (6.9)	HBV 0.6 HCV 1.0	0.439 0.318
	No	171	25 (14.2)		
FHHI	Yes	257	24 (9.5)	HBV 0.6 HCV 1.0	0.439 0.317
	No	149	17 (10.9)		

Table 4. T-test for AST and ALT among the pregnant women tested. Significance = P < 0.05, S.D. = Standard deviation, AST = Aspartate Transaminase, ALT = Alanine Transaminase.

Group	Parameter	Status	Mean±SD(iu/L)	X ²	P-value
HBsAg	AST	Positive (n = 41)	12.16±0.29	258.562	0.00
		Negative (n = 365)	10.55±0.05		
	ALT	Positive (n = 41)	8.01±0.01		
		Negative (n = 365)	5.54±0.005		
HCV	AST	Positive (n = 5)	10.67±0.01	359.270	0.00
		Negative (n = 365)	10.01±0.09		
	ALT	Positive (n = 5)	4.01±0.01		
		Negative (n = 406)	5.71±0.05		

Table 5. T-test for serum albumin among the pregnant women tested. Significance = P < 0.05, S.D. = Standard deviation.

Group	Parameter	Status	Mean±SD(IU/L)	X ²	P-value
HBsAg	Albumin	Positive (n = 41)	30.60±2.75	258.562	0.00
		Negative (n = 365)	35.58±3.82		
HCV	Albumin	Positive (n = 5)	28.5±2.12	386.246	0.000
		Negative (n = 401)	35.55±3.7		

d'Ivoire [22]. My finding was in agreement with the research carried out in Sudan. There was a lower prevalence rate of 0.01% in the United Kingdom [21]. A prevalence of 1.3% of mix infection for HBV and HCV in the tested population was found to be high when compared to other studies carried out in other part of Nigeria. Ose *et al.* reported a 0.57% in south west Nigeria. This could be as a result of lack or poor public enlightenment.

This study also showed that out of 406 respondents, the highest prevalence for HBsAg were within the age group 15-24 years. In this study, pregnancy caused no alteration in the levels of the AST aminotransferase. Although the results of the aminotransferases assayed were normal in all subjects there was a significant increase in ALT and AST levels when HBsAg positive and negative pregnant subjects were compared. There was also a high frequency of HBsAg seropositivity as compared to HCV among pregnant women in this study and an increased serum AST and ALT level in the positive subjects than in the negative subjects. This is in line with the work of Hesper *et al.* [23] who detected increased ALT in association with HCV infection in primary care patients.

Furthermore, in this study, serum albumin was normal in both HBsAg and HCV negative pregnant women but slightly decreased in HBsAg and HCV positive pregnant women. This is in line with other studies that discovered that albumin level is normal in chronic hepatitis subjects but is usually decreased in acute hepatitis subjects.

The dangers inherent in the observed cases call for conscious effort to be addressed especially as it has been reported that infection acquired perinatally and in early childhood is usually asymptomatic but in the people who experience the disease, the severity of symptoms and illness vary widely [24-31].

Conclusion

The study revealed that HBV and HCV could be present in pregnant women and that both infections can co-exist in a pregnant woman. Also the study revealed that these viruses can alter liver serum albumin and liver enzymes (ALT and AST). There is therefore urgent need for free screening of all pregnant mothers for HBV and HCV. In addition, there should be robust immunizations of all HBsAg negative pregnant women and their infants against HBV. The study also showed a high prevalence of HCV in pregnant women studied.

In conclusion, therefore both HBV and HCV can be present in pregnant women and can alter liver amino transferases and serum albumin. These liver damage markers were not indicated in the pregnant women studied.

References

1. Madhava V, Burgess C, Drucker E (2002) Epidemiology of chronic hepatitis C virus infection in sub-Saharan Africa. *Lancet Infect Dis* 2: 293-302. [Crossref]
2. Kramvis A, Kew M, François G (2005) Hepatitis B virus genotypes. *Vaccine* 23: 2409-2423. [Crossref]
3. Goldstein ST, Zhou F, Hadler SC, Bell BP, Mast EE, et al. (2005) A Mathematical model to estimate global hepatitis B disease burden and vaccination impact. *Int J Epidemiol* 34:1329-1339.
4. Pungpapong S, Kim WR, Poterucha JJ (2007) Natural history of hepatitis B virus infection: an update for clinicians. *Mayo Clin Proc* 82: 967-975. [Crossref]
5. Samuel D, Muller R, Alexandre G (2004) Educational Research, National Hepatitis B Virus Programme. *Infectious Diseases* 234:221-332.
6. Polyak P (2006) Innate intracellular defence against HIV and its modulation by HCV gene product. Postgraduate Course, Vienna pp: 30-33.
7. Obi RK, Umeh SC, Okurede OH and Iroagba II (2006) Prevalence of Hepatitis B virus infection in an antenatal clinic in Portharcourt, Nigeria. *African Journal of Clinical and Experimental Microbiology* 7:78-82.

8. Dienstag JL (1993) Hepatitis B virus infection. *N Engl J Med* 14: 1486-1500.
9. Yakasai IA, Ayyuba R, Abubakar IS, Ibrahim SA (2012) Seroprevalence of hepatitis B virus infection and its risk factors among pregnant women attending antenatal clinic at Aminu Kano Teaching Hospital, Kano, Nigeria. *J Basic C1iu Reprod Sci* 1:49-55.
10. Oladeinde BH, Omoregie R, Oladeinde OB (2013) Prevalence of HIV, HBV and HCV infections among pregnant women receiving antenatal care in a traditional birth home in Benin City, Nigeria. *Saudi Journal for health science* 2:113-117.
11. Ganem D, Schneider RJ (2001) Viruses and their replication (4th Edn) 56: 2923-2969.
12. Abdool Karim SS, Thejpal R, Singh B (1989) High prevalence of hepatitis B virus infection in rural black adults in Mseleni, South Africa. *Am J Public Health* 79: 893-894. [Crossref]
13. Zali R, Mohammad K, Farhadi A (1996) Epidemiology of hepatitis B in the LR of Iran. *EMHJ* 2:290-298.
14. WHO (1999) Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the viral Hepatitis Prevention Board, Antwerp. *Belgium Journal of Viral Hepatology* 6:35-47.
15. Uneke CJ, Ogbu O, Inyama PU, Anyanwu GI, Njoku MO, et al. (2005) Prevalence of hepatitis-B surface antigen among blood donors and human immunodeficiency virus-infected patients in Jos, Nigeria. *Mem Inst Oswaldo Cruz* 100: 13-16. [Crossref]
16. Ndams IS, Joshua LA, Luka SA, Sadiq HO (2008) Epidemiology of Hepatitis B infection among Pregnant Women in Minna. *Nigeria Science World Journal* 3:5-8.
17. Baba MM, Onwuka IS, Baba SS (1999) Hepatitis B and C virus infections among pregnant women in Maiduguri, Nigeria. *Central European Journal of Public Health* 7:60-62.
18. Mbaawuaga EM, Enenebeaku MN, OkopiJA, Damen JG (2008) Hepatitis B Virus (HBV) Infection among Pregnant Women in Makurdi, Nigeria. *African Journal of Biomedical Research* 11:155-159.
19. Onakewhor JUE, Okonofua FE (2008) Seroprevalence of Hepatitis B surface antigen (HBsAg) in a tertiary health facility in Nigeria. *Journal of Obstetrics and Gynaecology* 21:583-586.
20. Luka SA, Ibrahim MB, Iliya SN (2008) Seroprevalence of hepatitis B surface antigen among pregnant women attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. *Nigerian Journal of Parasitology* 29: 38-41.
21. Wesley J, Kieter, Alter, Belbin L (2000) Risk of hepatitis B transmission and breast-fed infants of chronic hepatitis B carriers.
22. Zuccotti J, Johann D, Harald H (2006) Australian Consensus-Statement for diagnosis and therapy of Hepatitis B.
23. Hesper C, Van Essen G, Frijling BD, De Wit NJ (2012) Follow-up of mild Alanine aminotransferase elevation identifies hidden hepatitis C in Primary Care. *Br J Gen Pract* 62: 212-216.
24. Akani CI, Ojule AC, Oporum HC, Ejilemele AA (2005) Sero-prevalence of hepatitis B surface antigen (HBsAg) in pregnant women in Port Harcourt, Nigeria. *Niger Postgrad Med J* 12: 266-270. [Crossref]
25. Alter MJ (1996) Epidemiology of hepatitis C. *Eur J Gastroenterol Hepatol* 8: 319-323. [Crossref]
26. Hill JB, Sheffield JS, Kim MJ, Alexander JM, Sercely B, et al. (2002) Risk of hepatitis B transmission in breast-fed infants of chronic hepatitis B carriers. *Obstet Gynecol* 99: 1049-1052. [Crossref]
27. Lin HH, Kao JH, Hsu HY, Ni YH, Yeh SH, et al. (1994) Possible role of high-titer maternal viremia in perinatal transmission of hepatitis C virus. *J Infect Dis* 169: 638-641. [Crossref]
28. Tse KY, Ho LF, Lao T (2005) The impact of maternal HBsAg carrier status on pregnancy outcomes: a case-control study. *J Hepatol* 43: 771-775. [Crossref]
29. Flynn J, Slovic P, Mertz CK (1994) Gender, race, and perception of environmental health risks. *Risk Anal* 14: 1101-1108. [Crossref]
30. Raynard B, Balian A, Fallik D, Capron F, Bedossa P, et al. (2002) Risk factors of fibrosis in alcohol-induced liver disease. *Hepatology* 35: 635-638. [Crossref]
31. Willey JM, Sherwood LM, Woolverton CJ, Presscott Harley, Kleins (2008) Microbiology (4th Edn) McGraw Hill Publishers, New York pp: 936-972.

Copyright: ©2017 Onwui FC. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.