OPEN ACCESS
Saudi Journal of Biomedical Research
Abbreviated Key Title: Saudi J Biomed Res
ISSN 2518-3214 (Print) |ISSN 2518-3222 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Prevalence and Frequency of Risk Factors in MDM2 Gene Associated with Liver Cancer among Patients in UCTH, Calabar, Nigeria

Sunday Cecilia James[1*], Kooffreh Mary Esien[1], Umoyen Anthony John[1], Ubi Godwin Micheal[1], Ekerette Ekerette Emmanuel[1], Ekpeyong Blessing Bassey[1]

[1]Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Calabar, Calabar – Nigeria

DOI: 10.36348/sjbr.2022.v07i08.002 | **Received:** 04.07.2022 | **Accepted:** 11.08.2022 | **Published:** 25.08.2022

***Corresponding author:** Sunday Cecilia James
Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Calabar, Calabar – Nigeria

Abstract

Liver cancer is a global health concern with high morbidity and mortality. It remains the sixth most common cancer worldwide despite the preventable nature of its risk factors. Mouse double minute-2 (*MDM2)* is one of the candidate genes associated with liver cancer and an over expression of the gene may lead to liver cancer. This research was aimed at investigating the prevalence and frequency of risk factors in the *MDM2* gene among liver cancer patients in the University of Calabar Teaching Hospital, Calabar. Forty five liver cancer patients and fifty controls were recruited for the study. Demographic information was collected from all the subjects using questionnaires and analyzed while blood samples were collected for molecular analysis. The result revealed a significant difference in the distribution of demographic variables in cases and controls ($P<0.05$). The overall frequency of mutations in *MDM2* gene in the studied population in Calabar was 40%, odd ratio 1.25 and sex ratio 2.2:0.5.The major risk factors of liver cancer are hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, heavy alcohol consumption, aflatoxin B1 ingestion, tobacco smoking and non-alcoholic fatty liver disease caused by obesity and insulin resistance.The risk factors associated with liver cancer cases revealed that Hepatitis C virus infection was detected in 11 patients (24.4%), Hepatitis B virus surface antigen positive ($HBsAg^{+}$) was found in 27 patients (60%), co-infection was found in 11 patients (24.4%), 19(42.2%) were involved in alcoholism and chain smoking, alcoholic consumption was 41(91%), consumption of moldy groundnut soup was 43 (95.6%) and family history of the disease was 10 (22.2%).The results revealed that out of the 45 patients recruited for the study, 15.1% were HIV positive, 17.7% were diabetic, 31.1% had melanin stool 24.4% had ascitis and 17.7% had hepatic encephalopathy as shown in Table 3. The liver function test carried out on the patients revealed that the albumin was 43.58±9.42 g/dl, creatinine was 136.62±177.98µmol/l, urea was 4.20±1.88mmol/l, the platelet was 180.74±104.70 $x10^9$ l/cells, the haemoglobin was 11.01±3.36g/dl, the alpha-fetoprotein (AFP) present was 398.8±103.2ng/ml, the aspartate aminotransferase (AST) was 45.65±66.01µmol/l, alanine transaminase was 31.67±33.37µmol/l and the international normalized ratio (INR) was 6.4±23.04.The alpha-feto protein among patients was 398.8±103.2ng/ml and this showed a strong indicator of hepatocellular cancer.The study therefore recommends that people in the area should avoid predisposing and risk factors such as alcoholism, smoking and unhealthy lifestyle which promotes the spreading of liver cancer among genetically (MDM2) susceptible liver cancer patients in Calabar The findings serve as baseline information for further studies on the genetic etiology and therapeutic initiative for the disease.

Keywords: Liver cancer, Hepatitis, liver disease, odd ratio, occurrence, alcoholism, smoking.

1. INTRODUCTION

Liver cancer is a global public health concern with mortality and morbidity on the rise (Okeke *et al.*, 2019; Ndom *et al.*, 2019). It remains the sixth most common cancer worldwide despite the preventable nature of its risk factors in Africa (Okeke*et al.*, 2019). Hepatocellular carcinoma (HCC) is commonly known as cancer of the liver (Nwokediuko *et al.*, 2011; Chen *et al.*, 2018;Mak *et al.*, 2018) and the most common form of liver malignancy among humans globally (Chia *et al.*, 2019). Hepatocellular carcinoma is a major cause of cancer mortality and morbidity in numerous parts of the world (Rasheed *et al.*, 2020). The etiology and pathogenesis of liver cancer is multifactorial and multi-step suggesting that its origin is as a result of different factors including genetic and environmental factor which is similar to other cancers (Roshani *et al.*, 2017;

Kulik and El-Serag, 2019; Lombardo *et al.,* 2020; Malu *et al*., 2020).

The major risk factors of HCC are hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, heavy alcohol consumption, aflatoxin B1 ingestion, tobacco smoking and non-alcoholic fatty liver disease caused by obesity and insulin resistance (Lovet *et al*., 2021). Hepatitis C virus (HCV) infection varies from asymptomatic infection to cirrhosis and hepatocellular carcinoma (Itskowitz, 2007; Mohd *et al.,* 2013; Forbi *et al.,* 2015). Most HCV infections remain asymptomatic for years (Koff, 2014; Sulkowski *et al.,* 2014). In Nigeria, chronic hepatitis C virus infection may progress to chronic hepatitis and cirrhosis; if not properly treated, leading to severe complications including HCC and death (Laraba *et al*., 2010; Kooffreh-Ada *et al*., 2016).

The complex multistep process of liver carcinogenesis or HCC tumorigenesis includes inflammation, hepatic damage, cirrhosis, increased liver fibrosis and finally hepatocellular carcinoma (Arzumanyan *et al.,* 2013; Tornesello *et al.,* 2016). Hepatocellular carcinoma (HCC) development are triggered by somatic and sporadic mutations occurring within an individual cell after conception and may not be present in the previous generation (Rice and Hernandez, 2019; Jiao *et al*., 2018; Lombardo *et al.,* 2020). Triggered mutations directly promote tumor growth; with variations between different types of cancers, but tend to occur early in the disease development (Roshani *et al*., 2017; Gomez *et al.,* 2018; Casuscelli *et al.,* 2019). The pathogenesis from healthy liver to hepatocellular carcinoma can be caused by different etiologic agents (chronic hepatitis B or C virus infections, diet containing aflatoxins, metabolic diseases among others) (Forbi *et al.,* 2015; Kooffreh-Ada *et al*., 2016). These etiologic agents can promote chronic hepatitis, which can progress to cirrhosis (Forbi *et al.,* 2015; Kooffreh-Ada *et al*., 2016). This progression is associated with genetic instability. Cirrhosis precedes hepatocellular carcinoma in around 90% of patients and contains areas of abnormal hepatocytes known as dysplastic foci (<1mm) (Rice and Hernandez, 2019). Thus, this paper seeks to reveal the prevalence and frequency of risk factors associated with MDM2 gene causing liver cancer among patients in University of Calabar Teaching Hospital in Calabar, Nigeria.

2. MATERIALS AND METHODS

2.1 The Study Location

The study was carried out at the Department of Internal Medicine, Gastroenterology and Hepatology Unit, University of Calabar Teaching Hospital, Calabar, Nigeria from 2020 to 2021.

2.2 Ethical Approval

Ethical approval was obtained from the University of Calabar Teaching Hospital Ethical Review Committee before the commencement of this study.

2.3 The Study Population

A total of 55 participants were recruited for the study; comprising 45 cases from clinically diagnosed liver cancer patients and 10 aged-matched controls who were all Nigerians.

2.4 Inclusion Criteria

The 45 liver cancer patients (hepatocellular carcinoma, liver cirrhosis) were diagnosed by the clinician based on either triphasic CT scans, positive histologic findings of focal lesions or an elevated alpha feto-protein (AFP) level (greater or equal to 400 ng/ml), combined with at least one positive image on sonography, and/or high-resolution contrast computed tomography. Hepatocellular carcinoma cases that were newly diagnosed, previously untreated (neither chemotherapy nor radiotherapy) and free from any other cancer were included in this study. The absence of hepatocellular carcinoma was established by the absence of focal lesions on the triphasic CT scan and by an elevated alpha-fetoprotein level (400ng/ml).

2.5 Exclusion Criteria

In the control, any possible history of hepatocellular carcinoma was excluded and subjects of non-African descendant were not recruited in the study.

2.6 Subject Enrolment and Sample Collection

Informed consent was obtained from the patients, guardian or parent before data collection and sample collection was initiated. Detailed medical history, age, race or ethnicity, social history/risk factors like alcohol intake, multiple sex partners/unprotected sex, dietary habits and family history of liver cancer case(s) was collected from the subjects recruited for this study. 2-3ml of whole blood was collected from both patients and controls, put into EDTA bottle and labeled properly. Blood samples was stored in deep freezer (-20^0c) before onward transportation to the virology and molecular diagnostic units, International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria for molecular analysis.

2.7 Statistical Analysis

The socio-demographic variables and clinical data were computed and analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Continuous variables were compared between liver cancer patients and controls using student *t*-test.

3. RESULTS

Prevalence and socio-demographic studies on liver cancer patients (hepatocellular carcinoma and liver Cirrhosis) in University of Calabar Teaching Hospital (UCTH) was carried out using 35 HCC cases, 10 liver cirrhosis patients with or without hepatitis viral infections, and 50 controls. The patients consisted of 31

(68.9%) males and 14(31.1%) females with mean age of cases being 41.51±2.13 years as shown in Table 1. In the HCC cases, the sex ratio was 2.2:0.5 and the odds ratio was 1.25. The male and female patients were 25(55.5%) and 10(22.2%) for the HCC, 7(15.6%) and 3(6.7%) for the liver cirrhosis. The healthy controls consisted of 6(60%) males and 4(40%) females with the mean age of 39.16.28±2.11 (Table 1).

The patients were found to be predominantly from the Northern and Central part of Cross River with 66.4% comprising of Yakurr (22.2%), Boki and Ogoja (13.3%) each, Obubra (4.4%) and Yala (2.2%). Efik was (20%), Annang (8.8%), Ibibio (2.2%) and Igbo (2.2%) from southern part of Cross River, AkwaIbom and Abia State (Table 1). The educational status of patients for tertiary, secondary and primary school were 66.7%, 26.7% and 6.7% respectively while the marital status of patients were 33(73.3%), 10(22.2%) and 2(4.4%) for married patients, single and widowed respectively (Table 1). There was a significant difference P< 0.05 in the distribution of demographic variables in cases and control (Table 1).

Table 1: Prevalence and Socio-demographic characteristics of subjects

Variables	Distribution in cases (%) (n=45)	Controls (%) (n=10)	Odd ratio	P-value Cal	P- value Tab
Sex				3.79	0.37
Male	31(68.9)	6(60)	1.25		
Female	14(31.1)	18(40)	(0.30-2.20)		
Age (years)					
Mean age	41.51±2.13	39.16±2.11			
Type of liverdisease				4.72	1.91
HCC	35(77.8)	0			
LC	10(22.2)	0			
Ethnicity				2.01	1.31
Yakurr	10(22.2)	2(20)			
Boki	6(13.3)	1(10)			
Yala	1(2.2)	1(10)			
Ogoja	6(13.3)	1(10)			
Obubra	2(4.4) = 66.4%	1(10)			
Obudu	2(4.4)	0(0)			
Biase	1(2.2)	1(10)			
Akamkpa	2(4.4)	0(0)			
Efik	9(20.0)	1(10)			
Anang	4(8.8)	0(0)			
Ibibio	1(2.2)	1(10)			
Igbo	1(2.2)	1(10)			
Educational status				1.12	0.52
Tertiary	30(66.7)	3(30)			
Secondary	12(26.7)	5(50)			
Primary	3(6.7)	2 (20)			
Marital Status				2.21	0.90
Married	33(73.3)	3(30)			
Single	10(22.2)	6(60)			
Widowed	2(4.4)	1(10)			

The risk factors associated with liver cancer cases are presented in Table 2. Hepatitis C virus infection was detected in 11 patients (24.4%), Hepatitis B virus surface antigen positive ($HBsAg^+$) was found in 27 patients (60%), co-infection was found in 11 patients (24.4%), 19(42.2%) were involved in alcoholism and chain smoking, alcoholic consumption was 41(91%), consumption of moldy groundnut soup was 43 (95.6%) and family history of the disease was 10 (22.2%).

Table 2: The risk factors for liver diseases in UCTH, Calabar

Variables	Total (%) n=45
HCV	11 (24.4)
$HBsAg^+$	27 (60)
$HCV/HBsAg^+$	11 (24.4)
$HBeAg^+$	7 (15.6)
Alcoholism and smoking	19 (42.2)
Alcoholic consumption	41 (91)
Smoking	16 (35.6)
Consumptionsof mouldy groundnuts	43 (95.6)
Family history	10 (22.2)
Use of sharp objects	32 (71.1)

The results revealed that out of the 45 patients recruited for the study, 15.1% were HIV positive, 17.7% were diabetic, 31.1% had melanin stool 24.4% had ascitis and 17.7% had hepatic encephalopathy as

shown in Table 3. The liver function test carried out on the patients revealed that the albumin was 43.58±9.42 g/dl, creatinine was 136.62±177.98µmol/l, urea was 4.20±1.88mmol/l, the platelet was 180.74±104.70 x10^9 l/cells, the haemoglobin was 11.01±3.36g/dl, the alpha-fetoprotein (AFP) present was 398.8±103.2ng/ml, the aspartate aminotransferase (AST) was 45.65±66.01µmol/l, alanine transaminase was 31.67±33.37µmol/l and the international normalized ratio (INR) was 6.4±23.04.

Table 3: Clinical and liver function test of patients

Variable	Total (n=45)
HIV positive	7 (15.1)
Diabetic cases	8 (17.7)
Melena stool	14(31.1)
Ascitis	11 (24.4)
Hepatic encephalopathy	8 (17.7)
Albumin (g/dl)	43.58±9.42
Creatinine (µmol/l)	136.62±177.98
Urea (mmol/l)	4.20±1.88
Platelet (x10^9 l/cells)	180.74±104.70
Hb (g/dl)	11.01±3.36
AFP (ng/ml)	398.8±103.2
AST (µmol/l)	45.65±66.01
ALT (µmol/l)	31.67±33.37
INR	6.4±23.04

AFP: Alpha-feto protein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; INR: International normalized ratio; HIV: Human immuno-deficiency virus; Hb: Haemoglobin, Normal range for liver function test:
AFP – 10ng/mL – 20ng/mL, AST - 8µmol/L - 33µmol/L, ALT- 7µmol/L -56µmol/L

DISCUSSION

The frequency of 40% for *MDM2* gene mutations was observed in this study among liver cancer patients in Calabar which is similar to the frequency of SNP309 G allele of *MDM2* reported as 33% in European Caucasian but in contrast with the African American population with a frequency of 11% (Bond *et al.*, 2004). Similarly in Egypt, the frequency of the mutant allele of *MDM2* was 32.5 (Zaky *et al*., 2020), in Korea, the frequency of *MDM2* was 40.8% (Yoon *et al*., 2008), in Japan, the frequency was 33% (Dharel *et al*., 2006) and was 35.63% in china (Su *et al*., 2014) which were similar to the frequency in our present study. In contrast, there was a higher frequency of 52.5% in Italy (Di Vuolo *et al*., 2011).The frequency of 40% of the *MDM2* gene mutations in liver cancer patient in Calabar may be associated with consumption of food heavily contaminated by aflatoxin B1 and HBV/HCV co-infections endemic in Northern and Central Cross River State. Groundnut has been reported to be the most heavily contaminated food by aflatoxin (Atawodi *et al*., 1993). Consumption of mouldy groundnut is one of the local delicacies of the natives of Northern/Central Cross River State, and these may be attributed to the cause of HCC among the studied population in Calabar. Researchers have associated the amount of groundnut ingested daily with significant levels of serum aflatoxins to the etiology of HCC (Kirk *et al*., 2005).

The odds ratio of 1.25 was recorded in this study in Calabar and similar odds ratio of 1.36 which was documented in Egyptian population (Zaky *et al*., 2020), but higher odds ratio of 2.0 reported in Ibadan in the molecular study of *p53* which is another candidate gene in the etiology of liver cancer (Igetei *et al*., 2008) and 2.461 documented in Mansoura city in another Egyptian population (Neamatallah *et al*., 2014). The reason for differences in the prevalence of the disease in different studied population may be due to the sample size, differences in the patients exposure to risk factors and age/or gender characteristics of participants enrolled in the study. These reasons are similar to those reported in previous studies (Igetei *et al*., 2008; Neamatallah *et al*., 2014; AbdElhameed *et al*., 2018).

The risk factors that may be associated with liver cancer in present study in Calabar are HBV, HCV, alcoholism, smoking and consumption of diets contaminated with aflatoxins. Risk factors are documented in USA (Sun *et al*., 2021), African Populations (Yang *et al*., 2016; Mak *et al*., 2018; Petrick *et al*., 2020), also in Zaria, Nigeria (Bello and Borodo, 2019), Enugu (Nwokediuko *et al*., 2013; Nwokediuko *et al*., 2011), Bayelsa state (Ochei *et al*., 2016; Buseri *et al*., 2010), Rivers State (Seleye-Fubara and Jebbin, 2007), in Calabar and Lafia(Uchenna *et al*., 2016). Hepatitis B and hepatitis C coinfections was previously reported as a risk factors in Calabar by researchers (Kooffreh-Ada *et al*., 2016) among chronic liver disease subjects which are similar to the results of this present research in Calabar. The main risk factors for disease presentation in this research were HBV,

HCV and diets which agrees with other studies in Italy (Tornersello *et al*., 2013; Di-Vuolo *et al*., 2011; Lombardo *et al*., 2020), Turkey (Ozdemir *et al.*, 2010), Egyptian populations (Elsamanoudy *et al*., 2012; Neamatallah *et al*., 2014; AbdElhameed *et al*., 2018; Zaky *et al.*, 2020). The same risk factors mentionedabove are also reported in Morocco (Ezzikouri *et al*., 2011), Kenyan Population (Ochwoto *et al.*, 2019; Oduma *et al.*, 2020) and Ibadan (Igetei *et al.*, 2008) all among liver cancer patients.

In Naples, Italy, *MDM2* polymorphism and risk of HCC was assessed using 61 cases of HCC and 122 controls. The findings revealed 47.5% and 52.5% for A alleles and G alleles respectively with an odd ratio of 1.89 (Di Vuolo *et al.*, 2011).The TT, TG, GG and GG+TG genotype frequency were 21.3%, 52.5%, 26.2% and 78.7% respectively (Di Vuolo *et al.*, 2011).

The result revealed that the frequency of TT, GT and GG genotypes were 21.52%, 49.95% and 28.45% respectively (Qiu *et al.*, 2016). In Xiamen, China, the association between polymorphism in tumour suppressor gene and oncogene and risk of HCC was assessed using 160 cases and 160 aged controls. The result revealed that the frequency for TT genotype was 50% and GG genotype was 35.63% (Su *et al.*, 2014). Similarly, Wang *et al.*, 2013 carried out a study on the association of *MDM2* with the risk of hepatocellular carcinoma using 286 cases and 375 controls. The result showed that the frequencies of TT, GT and GG genotypes were 19.93%, 46.15% and 33.92%. In another study carried out in Shanghai, china. The influence of the combined effect of *p53* and *MDM2* polymorphisms on the risk of developing HCC was investigated using 350 cases and 326 controls. The result showed that the frequency of TT genotype was 25.4%, GT genotype was 50.3% and TT genotype was 24.3%. The G/G genotype of *MDM2* was associated with increased risk of developing HCC (Yang *et al.*, 2013). In Nanjing, China, the association between single nucleotide polymorphism in *MDM2* and the susceptibility of hepatocellular carcinoma was investigated using 166 cases. The findings revealed that the frequency of TT, TG, GG, TG+GG and TT+TG were 19%, 63.8%, 17.2%, 81% and 82.8% in cases respectively (Leu *et al.*, 2009).

In Tokyo Japan, a study was conducted by Dharel *et al.*, (2006) to show that *MDM2* SNP309 polymorphism is associated with hepatocellular carcinoma and 48 healthy subjects was used for the study. The result revealed that the frequency of TT, GT and GG were 16.6%, 50.8% and 33% respectively. The proportion of G/G genotype of the SNP309 in patient with HCC (33%) was significantly higher than that in patient without HCC (23%) with an odd ratio of 2.28.

CONCLUSION

With recent genetic and molecular advances, it has become pertinent for medical practitioners to diagnosed disorders and diseases at the molecular level to aid in proper clinical diagnosis. Following the various analyses performed in this study, Findings revealed that there was high prevalence with high frequencies of risk factors inducing liver cancer in the area. The study therefore recommends that people in the area should avoid predisposing and risk factors such as alcoholism, smoking and unhealthy lifestyle which promotes the spreading of liver cancer among genetically (MDM2) susceptible liver cancer patients in Calabar and this serve as baseline information for further studies on the genetic etiology of the disease.

REFERENCES

- AbdElhameed, A. H., Abo-Elenein, A. M., Ibrahim, W. S., El-kassas, G. M., & Noweir, M. A. (2018). Study of P53 gene mutations as a new early diagnostic marker of hepatocellular carcinoma in Egyptian patients. *Madridge Journal of Oncogen*esis, 2(1), 21-29.
- Abudu, E. K., & Akinbami O. S. (2016). Histologic profile of primary gastrointestinal malignancies in Uyo City. *Rare Tumours*, 8(1), 6183.
- Arzumanyan, A., Reis, H. M., & Feitelson, M. A. (2013). Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nature Review in Cancer*, 13, 123-135.
- Bond, G. L., Hu, W., Bond, E. E., Robins, H., Lutzker, S. G., Arva, N. C., Bargonetti, J., Bartel, F., Taubert, H., Wuerl, P., Onel, K., Yip, L., Hwang, S-J., Strong, L. C., Lozano, G., & Levine, A. J. (2004). A single nucleotide polymorphism in the *MDM2* promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell*, 119, 591-602.
- Bosetti, C., Turati, F., & La, V. C. (2014).Hepatocellular carcinoma epidemiology. *Best Practice Research in Clinical Gastroenterology*, 28, 753-770.
- Buseri, F. I., Seiyaboh, E., & Jeremiah, Z.A. (2010).Surveying infections among pregnant women in the Niger Delta, Migeria.*Journal of Global Infectious Disease*, 2, 203-211.
- Candotti, D., Temple, J., Sarkodie, F. &Allain, J. P. (2003).Frequent recovery and broad genotype 2 diversity characterize hepatitis C virus infection in Ghana, West Africa. *Journal of Virology*, 77, 7914–7923.
- Cevik, D., Yildiz, G. &Ozturk, M. (2015).Common telomerase reverse transcriptase promoter mutations in hepatocellular carcinomas from different geographical locations.*World Journal of Gastroenterology*, 21(1), 311-317.
- Chen, J., Marechal, V. & Levine, A. J. (1993).Mapping of the p53 and mdm-2 interaction domains.*Molecular Cell Biology*, 13, 4107–4114.

- Chen, Y-L., Jeng, Y-M., Chang, C-N., Lee, H-J., Hsu, H-C., Lai, P-L.& Yuan, R-H. (2014). TERT promoter mutation in hepatocellular carcinomas: A strong association with hepatitis C infection and absence of hepatitis B infection. *International Journal of Surgery*, 12, 659-665.
- Chia, T.S., Wong, K. F. &Luk, J. (2019). Molecular diagnosis of hepatocellular carcinoma: trends in biomarkers combination to enhance early cancer detection. *Hepatoma Research*, 5:9-23.
- Dagogo-Jack, I. & Shaw, A. T. (2018).Tumor heterogeneity and resistance to cancer therapies.*Nature Review on Clinical Oncology*, 15, 81–94.
- Di Vuolo, V., Buonaguro, l., Izzo, F., Losito, S., Botti, G., Buonaguro, F. M. &Tornesello, M. L. (2011).TP53 and MDM2 gene polymorphisms and risk of hepatocellular carcinoma among Italian patients.*Infectious Agents and Cancer*, 6, 13-18.
- Duan, L., Yang, Q., Yang, J., Hu, Q., Wang, B., Li, P. & Chen, W. (2018).Identification of serum β-cateninas a biomarker in patients with HBV-related liver diseases. *Journal of Translational Medicine*, 16, 265-271.
- Duan, X. & Li, J. (2017). Association between MDM2 SNP309, p53 Arg72Pro and hepatocellular carcinoma risk a moose-compliant meta-analysis. *Medicine*, 96, 36-42.
- Ebughe, G. A., Inyama, M. A., Ugbem, T. I. &Ushie, D. E. (2019). The pattern of cancer cases in the rest of Cross River State not covered by Calabar cancer regiter between 2004- 2013. *Journal of Tropical Disease and Health*, 38(1), 1-12.
- Edamoto, Y., Hara, A., Biernat, W., Terracciano, L., Cathomas, G., Riehle, H. M., Matsuda, M., Fujii, H., Scoazec, J. Y. &Ohgaki, H. (2003). Alterations of *RB1*, *p53* and Wntpathwaysin hepatocellular carcinomas associated with hepatitis C, hepatitis B and alcoholic liver cirrhosis. *International Journal of Cancer*, 106(3), 334-341.
- Ezzikouri, S., El feydi, A. E., Afifi, R.., El kihal, L., Benazzouz, M., Hassar, M., Marchio, A., Pineau, P. &Benjelloun, S. (2009).*MDM2 SNP309T>G* polymorphism and risk of hepatocellular carcinoma: a case-control analysis in a Moroccan population. *Cancer Detection and Prevention Journal*, 32, 380-385.
- Fakharzadeh, S. S., Trusko, S. P. & George, D. L. (1991). Tumorigenic potential associated with enhanced expression of a gene that is amplified in a mouse tumor cell line. *The European Molecular Biology Organization Journal*, 10, 1565–1569.
- Forbi, J. C., Vaughan, G., Purdy, M. A., Campo, D. S., Xia, G. L., Ganova-Raeva, L. M., Ramachandran, S., Thai, H. &Khudyakov, Y. E. (2010). Epidemic history and evolutionary dynamics of hepatitis B virus infection in two remote communities in rural Nigeria.*Plos One*, 5, 11615-11629.
- Fusco, M., Girardi, E., Piselli, P., Palombino, R., Polesel, J., Malone, C., Scognamiglio, P., Pisanti, F. A., Solmone, M., Di Cicco, P., Ippolito, G., Franceschi, S. &Serraino, D. (2008). Epidemiology of viral hepatitis infections in an area of southern Italy with high incidence rates of liver cancer. *European Journal of Cancer*, 44(6), 847-853.
- Gomez, K., Miura, S., Huuki, L.A, Spell, B. S, Townsend, J.P. & Kumar, S. (2018).Somatic evolutionary timings of driver mutations.*Biomedical Central in Cancer*, 18, 85-92.
- Gouas, D. A., Villar, S., Ortiz-Cuaran, S., Legros, P., Ferro, G., Kirk, G. D., Lesi, O. A., Mendy, M., Bah, E., Friesen, M. D., Groopman, J., Chemin, I. & Hainaut, P. (2012). TP53 R249S mutation, genetic variations in HBX and risk of hepatocellular carcinoma in the Gambia.*Carcinogenesis*, 33(6), 1219-1224.
- He, F., Song, K., Guan, G., Huo, J., Xin, Y., Li, T., Liu, C., Zhu, Q., Fan, N., Guo, Y. &Wu, L. (2021).The Phenomenon of Gene Rearrangement is Frequently Associated with *TP53* Mutations and Poor Disease-Free Survival in Hepatocellular Carcinoma. *Pharmacogenomics and Personalized Medicine*,14 ,723–736.
- Ibrahim, I. A. &Pondei, K. (2014). Hepatitis B and C infection: should gynaecological patients be routinely screnned? *International Journal of Medicine and Biomedical Research*,3(1), 2-4.
- Inyang-Etoh, P. C., Aganyi, R. U., Agan, T. U.&Opara-Osuoha, U. (2016).Occurrence of Hepatitis B and C Viral infectionsamong pregnant women in Calabar, Cross RiverState, Nigeria.*Journal of Advances in Microbiology*, 1(1), 1-9.
- Inyang-Etoh, P. C., Eyo, G. O. & Philip-Ephraim, E. E. (2014).Occurrence of hepatitis 'B' and 'C' amongst patients on antiretroviral drug therapy (ART) in a treatment centre in Calabar, Nigeria. *International Journal of Medicine and Medical Sciences*, 6(6), 158-160.
- Jeannel, D., Fretz, C., Traore, Y., Kohdjo, N., Bigot, A., Pe- Gamy, E., Jourdan, G., Kourouma, K., Maertens, G. & other authors (1998). Evidence for high genetic diversity and long-term endemicity of hepatitis C virus genotypes 1 and 2 in West Africa.*Journal of Medical Virology*, 55, 92–97.
- Jedy-Agbaa, E., Curadob, M. P., Ogunbiyic, P., Ogaa, E., Fabowalec, T., Igbinobad, F., Osubord, G., Otue, T., Kumaie, H., Koechlinb, A., Osinubif, P., Dakuma, P., Blattnerg, W. &Adebamowo, C. A. (2012). Cancer incidence in Nigeria: A report from population-based cancer registries. *Cancer Epidemiology*, 36(5), 271–278.
- Jiao, J., Niu, W., Wang, Y., Baggerly, K., Ye, Y. & Wu, X. (2018).Prevalence of Aflatoxinassociated

TP53R249S mutation in hepatocellular carcinoma in Hispanics in South Texas.*Cancer Preventive Research*, 11, 103–112.

- Kancherla, V., Abdullazade, S., Matter, M. S., Lanzafame, M., Quagliata, L., Roma, G., Hoshida, Y., Terracciano, L. M., Ng, C. K. Y. &Piscuoglio, S. (2018). Genomic Analysis Revealed New Oncogenic Signatures in TP53-Mutant Hepatocellular Carcinoma. *Frontiers in Genetics*, 9, 2-16.
- Kooffreh, M. E., Udensi, O. U.&Umoyen, A. J. (2017). Modifying and adapting a plant-based extraction protocol for human genomic DNA extraction: a cost effective approach. *Global Journal of Pure and Applied Sciences*, 23, 1-4.
- Kooffreh-Ada, M., Okpokam, D. C., Okaormhe, Z. A. &Nna, V. U.(2016). Frequency of Hepatitis B and C Co-Infection in Chronic Liver Disease Patients in Calabar, Cross River State, Nigeria.*Nigerian Journal of Physiological Science*, 31, 043-047.
- Laraba, A., Wadzali, G., Sunday, B., Abdulfatai, O. &Fatai, S. (2010). Hepatitis C virus infection in Nigerians with chronic liver disease.*The International Journal of Gastroenterology*, 9, 17-24.
- Leu, J. D., Lin, I. F., Chen, S. M., Liu, C. C. & Lee, Y. J. (2009). Association between *MDM2-SNP309* and hepatocellular carcinoma in Taiwanese population.*World Journal of Gastroenterology*, 15(44), 5592-5597.
- Lovet, J. M., Kelley, R. K., Villanueva, A., Singal, A. G., Pikarsky, E., Roayaie, S., Lencioni, R., Koike, K., Zucman-Rossi, J. &Finn, R. S. (2021).Hepatocellular carcinoma.*Nature Reviews* (*Disease Primers*), 7(6), 1-28.
- Ma, R., Zhao, M., Zou, X., Zhou, J. &Bai, Z. (2021).MicroRNA polymorphism: A target for diagnosis and prognosis of hepatocellular carcinoma? (review). *Oncology Letters*, 21, 324-330.
- Mak, D., Babb de Villiers, C., Chasela, C., Urban, M. I. &Kramvis, A. (2018) Analysis of risk factors associated with hepatocellular carcinoma in black South Africans: 2000-2012. *Plos One*, 13(5), 1-14.
- Min, Q., Molina, L., Li, J., Michael, A. O. A., Russell, J. O., Preziosi, M. E., Singh, S., Poddar, M., Matz-Soja, M., Ranganathan, S., Bell, A. W., Gebhardt, R., Gaunitz, F., Yu, J., Tao, J. &Monga, S. P. (2019). Beta-catenin and Yes-associated protein 1 cooperate in hepatoblastoma pathogenesis. *The American Journal of Pathology*, 189(5), 1091-1104.
- Mohd, H. K., Groeger, J., Flaxman, A. D &Wiersma, S. T. (2013). Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology*, 57, 1333– 1342.
- Morounke, S. G., Ayorinde, J. B., Benedict, A. O., Adebayo, F. F., Adewale, F. O., Oluwadamilare, I., Sokunle, S. S. &Benjamim, A. (2017). Epidermiology and incidence of common cancers in Nigeria.*Journal of cancer Biology and Research*, 5 (3), 1105-1117.
- Ndom, P. (2019). Cancer prevention in Africa: liver cancer. *Electronic Cancer*, 13, 950 -958.
- Neamatallah, M. A., El-Missiry, M. A., Said, M.M.A., Elbendary, M., Othman, A. I. &Ammar, O.A. (2014). TP53 polymorphism as a risk factor for hepatocellular carcinoma in hepatitis C virus-infected Egyptian patients.*EgyptianJournal of Basic and AppliedSciences*, 1, 9-15.
- Njouom, R., Nerrienet, E., Dubois, M., Lachenal, G., Rousset, D.,Vessie`re, A., Ayouba, A., Pasquier, C. &Pouillot, R. (2007). Thehepatitis C virus epidemic in Cameroon: genetic evidence for rapidtransmission between 1920 and 1960. *Infection and GeneticEvolution*,7, 361–367.
- Nwafor, C. C. &Nwafor, N. N. (2018).The pattern and distribution of cancers in AkwaIbom State, Nigeria.*Niger Journal of Clinical Practices*, 21, 603-608.
- Nwokediuko, S. C., Osuala, P. C., Uduma, U. V., Alaneme, A. K., Onwuka, C. C. &Mesigo, C. (2013). Pattern of liver disease admissions in a Nigerian tertiary hospital.*Nigerian Journal of Clinical Practice*, 16(3), 399-342.
- Obiora, U. J. (2020).Cancer among women of reproductive age in Nigeria.*Asian Journal of MedicalSciences*, 11(3), 42-46.
- Ochei, K. C., Obeagu, E. I., Ibegi, B. T., Ugobo, E. I. &Odo, M. (2016). Merit Research Prevalence of hepatitis B and C from samples received from the various wards in Niger Delta University Teaching Hospital (NDUTH), Okolobiriforhaematological analyses. *Journal of Medicine and Medical Sciences*, 4(4), 204-209.
- Okeke, E., Davwar, P. M., Roberts, L., Sartorius, K., Spearman, W., Malu, A. &Duguru, M. (2019).Epidemiology of liver cancer in Africa: current and future trends. *Seminar in Liver Disease*, 39, 55-66.
- Okpokam D. C., Kooffreh-Ada M., Okhormhe Z. A., Akpabio, E. N., Akpotuzor, J. O. &Nna, V. U. (2015). Hepatitis D virus in chronic liver disease patients with hepatitis B surface antigen in University of Calabar Teaching Hospital, Calabar, Nigeria.*British Journal of Medicine and Medical Research*,6(3), 312-318
- Otto, T. &Sicinski, P. (2017).Cell cycle proteins as promising targets in cancer therapy.*Nature Review in Cancer*, 17, 93–115.
- Ozdemir, F. T., Tiftikci, A., Sancak, S., Eren, F. &Tahan, V. (2010).The Prevalence of the mutation in codon 249 of the P53gene in patients with hepatocellular carcinoma (HCC) in Turkey.*Journal of Gastro-intestinal Cancer*, 41(3), 185-189.

- Peng, Q., Mo, C., Qin, A., Lao, Q., Lao, X.,Chen, Z., Sui, j., Wu, J., Zhai, L., Yang, S., Qin, X. & Li, S. (2013). *MDM2* SNP309 polymorphism contributes to endometrial cancer susceptibility: evidence from a meta-analysis. *Journal of Experimental & Clinical Cancer Research,* 32, 85-89.
- Petrick, J. L., Florio, A. A.,Znaor, A., Ruggieri, D., Laversanne, M., Alvarez, C. S., Ferlay, J., Valery, P.C., Bray, F. &McGlynn, K. A. (2020). International trends in hepatocellular carcinoma incidence, 1978-2012. *International Journal of Cancer*, 147(2), 317–330.
- Pezzuto, F., Buonaguro, L., Buonaguro, F. M. &Tornesello, M. L. (2017).Frequency and geographic distribution of TERT promoter mutations in primary hepatocellular carcinoma.*Infectious Agents and Cancer*, 12(27), 1-9.
- Protzer, U., Maini, M. K. &Knolle, P. A. (2012).Living in the liver: hepatic infections.*Nature Review in Immunology*, 12, 201–213.
- Qiu, M., Liu, Y., Yu, X., Qin, L., Bei, C., Zeng, X., Qiu, X., Tang, B., He, S. & Yu, H. (2016).Interraction between *p53* codon 72 and *MDM2* 309T>G polymorphisms and the risk of hepatocellular carcinoma.*Tumour Biology,* 37(3), 3863-3870.
- Rasheed, W. M., Kandeil, M. A. M., Mahmoud, O. M. &Ezzat, S. (2020). Hepatocellular carcinoma (HCC) in Egypt: a comprehensive overview. *Journal of the EgypianNatonal Cancer Institute*, 32, 5-21.
- Raza, S. A., Clifford, G. M. &Franceschi, S. (2007). Worldwide variation in the relative importance of hepatitis B and hepatitis C viruses in hepatocellular carcinoma: a systematic review. *Britain Journal of Cancer*, 96, 1127–1134.
- Rice, A. & Hernandez, A. D. R. (2019).The mutational landscape of pancreatic and liver cancers, as represented by circulating tumor DNA.*Frontier Oncology*, 9(952), 1-18
- Ruggieri, A., Argentini, C., Kouruma, F., Chionne, P., D'Ugo, E., Spada, E., Dettori, S., Sabbatani, S. &Rapicetta, M. (1996).Heterogeneity of hepatitis C virus genotype 2 variants in West Central Africa (Guinea Conakry).*Journal of General Virology*, 77, 2073–2076.
- Samuel, N. & Hudson, T. J. (2011). The molecular and cellular heterogeneity of pancreatic ductal adenocarcinoma.*Nature Review in Gastroenterology and Hepatology*, 9, 77–87.
- Seleye-Fubara, D. &Jebbin, N. J. (2007). Hepatocellular carcinoma in Port Harcourt Nigeria: Clinicopathologic study of 75 cases. *Nigerian Journal of Clinical Practice*, 6 (2), 54-57.
- Shepard, C. W., Finelli, L. & Alter, M. J (2005).Global epidemiology of hepatitis C virus infection.*Lancet Infectious Diseases*, 5, 558–567.
- Su, Y-H., Lin, S. Y., Song, W. & Jain, S. (2014). DNA markers in molecular diagnostics for hepatocellular carcinoma. *Expert Review of Molecular Diagnostics*, 14(7), 803-817.
- Sun, J., Althoff, K. N., Jing, Y., Horberg, M. A., Buchacz, K., Gill, M. J., Justice, A. C., Kirk, G. D. & North American AIDS Cohort Collaboration on Research and Design of IeDEA. (2021). Trends in Hepatocellular Carcinoma Incidence and Risk among Persons with HIVin the US and Canada, 1996-2015.*Journal of Ameican Medical Association* (*JAMA*)*Network Open*, 4(2), 1-15.
- Tamura, K., Stecher, G., Peterson D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*, 2725-2729.
- Tornesello, M. L, Buonaguro, L., Izzo, F. &Buonaguro, F. M. (2016). Molecular alterations in hepatocellular carcinoma associated with hepatitis B and hepatitis C infections. *Oncotarget*, 7, 25087–25102.
- Totoki, Y., Tatsuno, K., Covington, K. R., Ueda, H., Creighton, C. J., Kato, M., Tsuji, S., Aburatani, H. &Shibata, T. (2014).Trans-ancestry mutational landscape of hepatocellular carcinoma genomes.*Nature Genetics*, 46(12), 1267-1273.
- Uchenna, O., Ruth, B., Ogbu, N. &Victor, N. (2016). Characteristics of hepatocellular carcinoma in two tertiary hospitals in Nigeria - has anything changed? *International Journal of Sciences*, 5(11), 43-48.
- Vhriterhire, R. A., Ngbea, J. A., Ojo, B. A., Jegede, O. O., Manasseh, A. N., Ayuba, M. D. &Mandong, B. M. (2016). Hepatocellular carcinoma sub-types in North-Central Nigeria: a histological review of liver biopsies. *British Journal of Medicine and Medical Research*, 12(4), 1-8.
- Wan, Y., Wu, W.,Yin, Z., Guan, P. & Zhou, B. (2011). *MDM2* SNP309, gene-gene interaction, and tumour susceptibility: an updated meta-analysis. *Biomedical Central Cancer*, 11, 208.
- Wang, X., Zhang, G. & Zhang, Y. (2013). Association between single nucleotide polymorphisms *MDM2* and the susceptibility of hepatocellular carcinoma.*Journal of Oncology*, 27, 1801-1804
- Wansbrough-Jones, M. H., Frimpong, E., Cant, B., Harris, K., Evans, M. R. &Teo, C. G. (1998).Prevalence and genotype of hepatitis C virus infection in pregnant women and blood donors in Ghana.*Translational Research in Sociology and Tropical Medical Hygiene*, 92, 496–499.
- Yang, Y., Xia, T., Li, N., Zhang, J., Yang, Y., Cong, W., Deng, Q., Lan, K. & Zhou, W. (2013). Combined effects of *p53* and*MDM2*

polymorphisms on susceptibility and surgical prognosis in hepatitis B virus-related hepatocellular carcinoma.*Protein cell*, 4(1), 71-81

- Yoon, Y. J., Chang, H. Y., Ahn, S. H., Kim, J. K., Park, Y. K., Kang, D. R., Park, J. Y., Myoung, S. M., Kim, D. Y., Chon, C. Y.& Han, K-H. (2008). MDM2 and p53polymorphisms are associated with the development of hepatocellularcarcinoma in patients with chronic hepatitis B virus infection. *Carcinogenesis*, 29(6), 1192–1196.
- Yu, B., O'Toole, S. A. & Trent, R. J. (2015).Somatic DNA mutation analysis in targeted therapy of solid tumours.*Translation and Pediatrics*, 4, 125–138.
- Zaky, A.M., Hanafy, S.M., El-Bordiny, M. M& El-Wafa, R. A. H. A. (2020). Association between TP53 and MDM2 Gene Polymorphisms and Risk of Hepatocellular Carcinoma in Hepatitis C Virus among Egyptian Populations.*Asian Journal of Research in Biochemistry*, 7(4), 148-156.
- Zhang, Y. & Lu, H. (2009). Signaling to p53: ribosomal proteins find their way. *Cancer Cell*, 16, 369–377.
- Zhao, Y., Zhu, C., Chang, Q., Yang, J., Liu, Y., Peng, P., Liu, C., Cheng, R., Chen, Z., Wu, Y., Cheng, L., Hu, L., Wu, X. & Jun, Yi. (2021). TP53 rs28934571 polymorphism increases the prognostic risk in hepatocellular carcinoma. *Biomarkers in Medicine*, 15(9), 615–622.