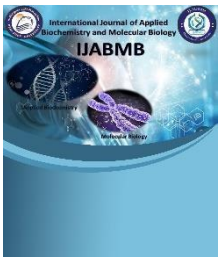
International Journal of Applied Biochemistry and Molecular Biology (IJABMB)



Evaluation of the micronucleus and comet assays in patients with hepatitis B in Samarra city/Iraq

Hanan Waleed Muhamed AL-SAMMARRAIE[*] Ph.D.

Department of Pathological Analysis, College of Applied Sciences, University of Samarra, Samarra, Iraq

***Corresponding author:**

Hanan Waleed Muhamed AL-SAMMARRAIE
Department of Pathological Analysis, College of Applied Sciences, University of Samarra, Samarra, Iraq,
Email: hanan.waleed.m@uosamarra.edu.iq

Tel: +905523651083

Running Title: ***Hepatitis B in Samarra city/Iraq***

Received on 1/11/2024

Revised on 21/11/2024

Accepted on 30/11/2024

Abstract

Background: Hepatitis B virus (HBV) is a major global health worries, impacting million of people globally.

Objective: Our goal in the present research was to investigate these types of tests and their applicability for evaluating patients' damage to genes. with HBV, and their potential implications for public health in the region.

Methods and patients: We employed the comet assay and MNi scoring to assess In contrast with normal controls, HBV-infected patients have higher levels of host genetic material in their oral and renal epithelial cells. The samples were collected from individuals (45 women and 51 men). Expert medical professionals have clinically determined that fifty healthy controls and forty-six HBV samples exhibit symptoms. They are between 15 and 80 years old.

Results: The findings showed that HBV patients had the greatest mean of mn (4.11), comparing to the healthy group of controls (0.26). The findings revealed that the mean BN within the HBV patient population was smaller (0.38) compared to the control group with no illness (3.61). In addition, the KL varied more in good-health controls (1.83) as opposed to people with illness (1.78). Moreover, the TCWD was higher among the HBV and healthy control groups. Furthermore, comparing the research groups with the wholesome groups as controls, there was not a noticeable distinction ($p \leq 0.05$).

Conclusion: The results of this study revealed that HBV is a serious cause of liver disease in Samarra.

Keywords

Hepatitis B virus, MNi, Samarra, TCWD.

Introduction

Hepatitis B virus (HBV) is a major world health worry, impacting numbers of people globally. The persistence of HBV infection can lead to severe complications, including liver cirrhosis and hepatocellular carcinoma. In Samarra City, Iraq, the prevalence of Hepatitis B has spurred research into various assessment methodologies that evaluate cellular damage, notably the micronucleus and comet assays. This article aims to investigate these tests and their significance in diagnosing genetic disorders among individuals with HEpatitis B with its possible impact on regional wellness. Hepatitis B, also known as hepatitis is an infection caused by viruses that targets the liver's cells, resulting with both acute and long-term illness. An ongoing infection with HBV can cause serious medical problems, such as liver inflammation, fibrosis, and a heightened risk of liver cancer (1). It is particularly prevalent in areas with inadequate vaccination coverage and limited access to healthcare, often putting populations at risk. In Samarra, like many regions in Iraq, the virus poses considerable challenges due to economic instability, limited healthcare resources, and varying levels of public awareness about the disease (2).

The micronucleus assay is a cytogenetic technique that detects chromosomal damage by identifying micronuclei—small, extra-nuclear bodies that form

when chromosomes or fragments thereof fail to migrate to daughter nuclei during cell division (3).

The presence of micronuclei can serve as an indicator of genotoxic stress, highlighting cellular responses to various factors, including viral infections like HBV. In patients with Hepatitis B, the micronucleus assay can reveal the extent of genomic instability induced by the virus. Research indicates that increased frequency of micronuclei correlates with viral load and liver dysfunction, suggesting a direct link between HBV infection and chromosomal damage (4). This assay offers a non-invasive means to monitor cytogenetic abnormalities, providing valuable insights into the disease's progression and potential complications. This comet test, also referred to as just one cell electrophoresis on gels, is an additional successful technique for detecting damage to DNA. This method allows researchers to evaluate the integrity of DNA within individual cells. When subjected to electrophoresis, damaged DNA strands move away from the nucleus, forming a "comet" tail that can be quantified. The length and intensity of the tail serve as indicators of DNA breakage. The comet assay's sensitivity makes it particularly effective for detecting early signs of DNA damage in HBV-infected patients. Studies have shown that individuals with chronic Hepatitis B exhibit higher levels of DNA strand breaks compared to healthy controls. This finding underscores the importance of monitoring DNA integrity as a marker for

estimating risk factors associated with HBV-related liver disease. While both the micronucleus and comet assays provide essential information regarding genetic damage, they have distinct advantages and limitations. The micronucleus assay excels at assessing chromosomal aberrations and can be performed using readily available peripheral blood samples. It reflects cumulative genetic damage over time but may not provide immediate insights into active DNA repair processes. Given the high prevalence of Hepatitis B in Samarra, integrating these assays into routine clinical practice could significantly improve patient management. By implementing the micronucleus and comet assays, healthcare providers can better stratify patients based on their level of genetic damage, leading to more personalized treatment approaches.

Moreover, these assessments can aid in determining prognosis and tailoring preventive strategies against HBV-related complications. In this investigation, we employed the comet assay and MNi scoring to assess HBV-infected oral as well as kidney epithelium cells show host-derived DNA production. patients in comparison to healthy controls.

Methods and patients

The sampling handle Between 15 January 2024 and 15 June 2024, 145 samples were collected from individuals (45 women and 51 men).

Expert medical professionals have clinically determined that fifty

healthy controls and forty-six HBV samples exhibit symptoms. They are between 15 and 80 years old.

Analysis of oral epithelial cells' micronucleus.

To ensure the good condition of the mouth tissues,, the patients performed examinations in the clinic under regular conditions and strong light. The exfoliated cells from scraping the oral mucosa collect in the following ways, per Alhamadany *et al*. [21]: A basic mouthwash produced The use of distilled water was utilized to minimize particulates. The internal sides of both the right as well as left cheeks were then lightly scraped with a water-soaked wooden spatula. These specimens have been set on two slides of glass and air-dried at room temperature. Next, as a gene toxicity examination, the After securing the film slides with solely methanol as well as letting them air dry, May Granwald the stain Giemsa was applied.

Assessment of damage to DNA in periphery lymphocyte and preparing the specimen

After being extracted using newly obtained plasma, the surrounding lymphocytes were spun and repeatedly cleaned using a buffer consisting of phosphate and saline solution. The test for viability It is necessary to conduct a viability study to determine the number of tests. material required for no less than 90% survival. To do this, mix 5 µl of trypan blue in combination and 10 l of 106 cells/ml using the tube of a microcentrifuge. After more than two minutes of standing, place it upon a platform and covered with

a cover slip. Determine the number of live (shiny) and expired (blue) cells throughout 100 the cells.

Alkaline comet assay

This test was carried out in accordance with the Trevigen protocol guidelines (Trevigen, Inc., Gaithersburg, US). The Lysis Solution was prepared and then allowed to cool at 4°C for at least 20 minutes. Next, The pipette 50 µl across Com et Slide TM and combine cells with 1 x 105/ml of melting low point of melting (LMA) agar. Utilizing the pipette tip's side, the agarose beads and cell were evenly spaced across the region. Following that, the film slides were kept straight and frozen in an unlit room at 4°C for an additional ten minutes. To increase sample adhesion in high-humidity conditions, the gelling period can be prolonged to 30 minutes.

Following thirty to sixty minutes, the presentations had been removed out of the 4°C Lysis Solvent as well as any leftover buffer were taken out of Com etSlide. For one hour at 4°C, a freshly prepared alkaline relaxing solution with a pH higher than 13 was added. The slides were put in the agarose slide trays with labels pointing regarding the black cathode after the alkali electrophoresis solution had been added. The current level of voltage was maintained at 21 volts for 30 minutes after the power source was set. The collected specimens subsequently gently submerged in water that was distilled twice, for five minutes all of them. additional electrophoresis solution twice,

and 70% alcohol (ethanol) once for five minutes. After 10 to 15 minutes of drying at 37 °C, the samples were examined. After applying 1X Ethidium Bromide stain on the CometSlideTM, any remaining stain was washed off with cold distilled water. The slides are then promptly graded. A fluorescent The quantity of damage to DNA was determined using an electron microscope that had a 40X lens and an electronic device to read the slides. The analytic technique was carried out on four com pictures and photos using 50 randomly selected cells, comprising 25 colonies on every plate. (3) investigated the velocity of movement each cell, the quantity of movement among damaged cells, evaluated the variety of migrated cells.

Results

Findings of the MNi assay on oral cell epithelial cells. Table 1 compares the levels of mn in mouth epidermal cells between HBV individuals and normal controls. The study found that HBV individuals had the greatest mn (4.11±0.724) when compared to normal controls (0.26±0.395). The HBV sufferers had a lower BN (0.38±0.149) compared the control group with no disease (0.46±0.783). See Table 1. The study found that healthy controls had a lower PN (0.49±0.327) compared to HBV infected (3.61±0.395). The study found healthy individuals had a smaller KR (4.26±1.092) than HBV patients (4.83±0.182). The study found that normal controls had a greater KL (1.83±0.721)

compared to HBV sufferers. (1.78±0.209). See Table 1.

Table 2 summarizes the findings of mn in epithelial cells of the mouth between HBV and normal controls by age group. The study found that HBV patients aged 2030 (4.81±0.328) had a substantially greater mean of mn compared to those aged 40-50 and 60-70 (4.17±0.424 and 4.09±0.481, respectively). Table 2 shows that those aged 50-60 had a median age of mn (1.29±0.437) in comparison with healthy controls. Furthermore, the probability distribution of the mn test measured in the kidneys epithelial cells amongst all research groups and the control group was clinically significant ($p < 0.05$).

Table 1: study groups' oral epithelial cells using the micronucleus test

Parameters / Study groups		Total mn	BN	PN	KR	KL	DIF
Healthy controls	Mean	0.26	0.46	0.49	4.26	1.83	92.7
	Std. Deviation	0.395	0.783	0.327	1.092	0.721	4.618
	No.	50	50	50	50	50	50
HBV patients	Mean	4.11*	0.38	3.61*	4.83	1.78	85.29
	Std. Deviation	0.724	0.149	0.395	0.182	0.209	0.305
	No.	46	46	46	46	46	46

The standard deviation is represented by S.E., and the t-test indicates that the standard deviation difference has significance at the 0.05 level. The entire micronucleus , or total mn BN: Binucleated DIF: Normal Differentiated Cell, KR: Karyorrhexis, KL: Karyolytic Cell, and PN: Pyknotic Nucleus

Results of the Comet assay in oral epithelial cells

Using a fluorescent microscope, the individual cell DNA movement patterns generated by this experiment are called "comets" because they resemble cometary stars (Fig. 1). 50–100 comets are typically assigned to each biological sample. See Figure 1.

Table 2: Total MNi of oral cell epithelial cells by age in study groups

Study groups	Healthy controls			HBV patients		
Statistical parameters / Age groups (Years)	Mean	Std. Deviation	No.	Mean	Std. Deviation	No.
10-20	0.29	0.398	14	-	-	-
20-30	0.17	0.295	20	4.81*	0.328	15
30-40	0.36	0.411	6	3.93*	0.259	14
40-50	0.49	0.321	4	4.17*	0.424	9
50-60	0.32	0.217	4	1.29	0.437	3
60-70	0.46	0.158	2	4.09*	0.481	5
Total	0.34	0.423	50	3.37*	0.385	46

Figure 1: Using the comet assay to demonstrate DNA damage tails

The results of the histological analysis for the total amount of epithelial cells in the mouth having DNA damage all through the study groups are shown in the figure.

Figure 2: TCWD in both the HBV disease subgroup and the unaffected control group 2. According to the current study, the HBV sufferers had a greater TCWD than the control group with no disease (Figure 2).

The comet test results for the Table 3 provides a summary of the total number of oral tissues with damaged DNA. Cell proliferation disparities among HBV and normal controls vary by age group. Results showed that the mean TCWD was substantially higher for the group of HBV patients aged 60-70 years (4.16±0.439) than for the 30-40 and 50-60 year demographic (3.84 ±0.326 and 3.27 ±0.422), significantly. However, the TCWD mean was smaller in the 20–30 range of age (2.75±0.299) compared to the healthy group in control. See Table 3.

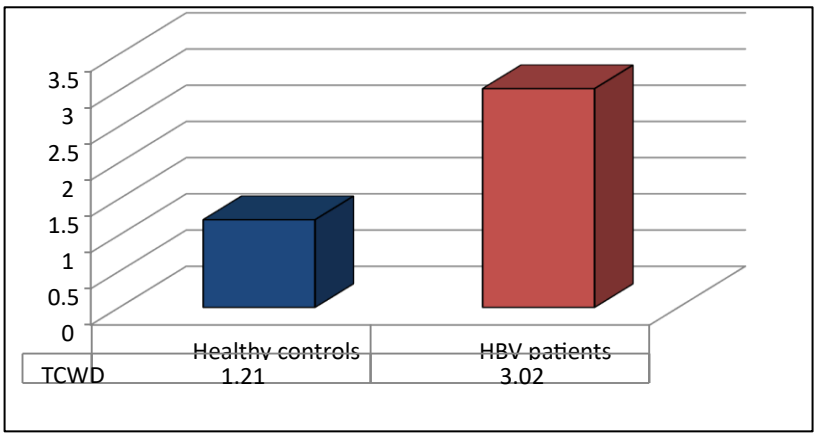


Figure 2. TCWD in both the healthy control population and the HBV patient population

Furthermore, TCWD evaluation in oral epithelial cells performed between the HBV and healthy groups of controls demonstrated clinical importance at $p < 0.05$, which is consistent with clinical relevance.

Table 3: Total percentage of oral epithelial cells with DNA damage by age group in the study groups

Study groups	Healthy controls			HBV patients		
Statistical parameters / Age groups (Years)	Mean	Std. Deviation	No.	Mean	Std. Deviation	No.
10-20	1.42	0.532	14	-	-	-
20-30	1.73	0.617	20	2.75*	0.299	15
30-40	1.44	0.398	6	3.84*	0.326	14
40-50	1.53	0.375	4	2.79*	0.379	9
50-60	1.74	0.486	4	3.27*	0.422	3
60-70	1.38	0.412	2	4.16*	0.439	5
Total	1.54	0.47	50	3.362*	0.373	46

Discussions

HBV infection is a public health issue. Even though Iraq has a 1% infection incidence, infection rates might rise in the absence of a successful preventative program. To avoid the negative effects of an HBV infection and to stop the virus from spreading, early identification is crucial. In this research, we examine the signs and symptoms that people with HBV suffer. Regretfully, most of the individuals who were enrolled in this research had no symptoms. To find such asymptomatic people, a robust screening procedure is necessary. The infection control department and health planners may also be able to restrict the spread of such an infection by identifying risk factors in our culture. Unsterilized dentistry and surgical instruments have previously been linked to blood-borne viral infection outbreaks in hospitals and private clinics (5). This could call for a careful examination of sterilization practices in all medical facilities, including dentistry offices. Moreover, those with a history of illicit intercourse and drug users are particularly vulnerable to contracting HBV. According to research done in Iran and Egypt, Of those who tested positive for HBsAg, 28% and 8.3%, correspondingly, had a history of drug use (6). However, 17.4% of the HBV-positive individuals in Iranian research reported having had extramarital sex in previous years (7). Further research in this field utilizing various data gathering techniques is required. Furthermore, HBV can be spread within the family

by toothbrushes, razors, and other household items (8). Some studies suggest that viral hepatitis causes somatic cells to become mutated. Chromosome instability may arise from multiple incorporation of the hepatitis C virus chromosome into the host's genome (9). The genetic makeup of other virus-carrying cells, such as red blood cells, are targeted by these viral DNA integration. in addition to the genomes of the host hepatocytes. According to Zondervan et al. (2000) (10), integrations encourage genetic recombination and, specifically, hepatocellular cancer. Recent studies have demonstrated that HBV infection causes genetic changes in somatic cells, including blood cells and hepatocytes, as indicated by an increase in chromosomal breakage (9). These investigations used cancer cell lines and hepatocytes (11). Few studies were able to identify a statistically significant impact by age or gender, despite the fact that many studies include the participants' age and gender. In two of the investigations, the mn frequency of buccal cells was greater in males than in women (12). The polluted environment of today exposes humans to a wide range of genotoxic chemicals.

According to Orta and Günebakan (2012)(13), aging has a major role when the big nuclear anomalies referred to as micronucleus occur (14)(15).

Conclusions

As demonstrated by higher micronucleus and comet testing findings in comparison to healthy controls, the study emphasizes the

substantial genetic damage seen in Hepatitis B patients in Samarra. These results highlight the necessity of improved public health efforts that tackle the consequences of cellular damage caused by HBV in the vicinity.

References

1. Varghese N, Majeed A, Nyalakonda S, Boortalary T, Halegoua-DeMarzio D, Hann H-W. Review of Related Factors for Persistent Risk of Hepatitis B Virus-Associated Hepatocellular Carcinoma. Cancers. 2024; 16(4):777. https://doi.org/10.3390/cancers16040777
2. Bashir Hamidu R, Hann RR, Hann H-W. Chronicles of HBV and the Road to HBV Cure. Livers. 2023; 3(2):232-239. https://doi.org/10.3390/livers3020015
3. Alhamadany A. Y. M. et al. Genotoxicity and genomic instability in oral epithelial cells of agricultural workers exposed to pesticides using micronucleus and comet assay in Nineveh, Iraq. Journal of Applied and Natural Science. 2023; 15(2), 473 - 479. https://doi.org/10.31018/jans.v15i2.4329
4. Leite ST, Silva MB, Pepato MA, Souto FJ, Santos RA, Bassi-Branco CL. Increased frequency of micronuclei in the lymphocytes of patients chronically infected with hepatitis B or hepatitis C virus. Mem Inst Oswaldo Cruz. 2014;109(1):15-20. doi: 10.1590/0074-0276140183. PMID: 24626305; PMCID: PMC4005534.

5. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine. 2012;30(12):2212-9. [PubMed ID: 22273662]. https://doi.org/10.1016/j.vaccine.2011.12.116.

6. Awadalla H. Risk Factors of Viral Hepatitis B among Egyptian Blood Donors. British Journal of Medicine and Medical Research. 2011;1(1):7-13. https://doi.org/10.9734/bjmmr/2011/127.

7. Hussein N R, and Daniel S. A Study of Hepatitis B Virus Associated Risk Factors in Patients Attending Hepatitis Unit in Duhok City, Iraq. Arch Clin Infect Dis. 2017;12(3):e62420. https://doi.org/10.5812/archcid.62420.

8. Tu H, Yu C, Tong W, Zhou C, Li R, Huang P, Wang Q, and Chang Y. Evaluation of the liver and blood micronucleus, and comet assay end points in a 14-day repeated-dose study with methyl carbamate and 1,3propane sultone. Mutagenesis. 2021;36(6), 401–406. https://doi.org/10.1093/mutage/geab034

9. Huang JM, Huang TH, Qiu HY, Fang, XW., Zhuang TG, Liu HX, Wang, YH, Deng LZ. and Qiu JW. Effects of hepatitis B virus infection on human sperm chromosomes. World J Gastroenterol. 2003; 9(4): 736740.

10. Zondervan P.E, Wink J, Alers J.C, Ijzermans J.N, Schalm S.W, De Man R.A. and Van Dekken H. Molecular

cytogenetic evaluation of virusassociated and non-viral hepatocellular carcinoma: analysis of 26 carcinomas and 12 concurrent dysplasias. J. Pathol., 2000; 192, 207-215.

11. Livezey K.W, Negorev D, and Simon D. Increased chromosomal alterations and micronuclei formation in human hepatoma HepG2 cells transfected with the hepatitis B virus HBX gene. Mutat. Res., 2002; 505, 63-74.
12. Ambroise MM, Kanchana, B. and Manjiri P. Predictive value of micronucleus count in cervical intra epithelial neoplasia and carcinoma. Turk Patolji Derg. 2013; 29:171–78.
13. Orta T, and Günebakan S. (2012). The effect of aging on micronuclei frequency and proliferation in human peripheral blood lymphocytes. Indian J Hum Genet. 18(1):95–100.
14. Almola A H, Alhamadany AY M, Haddad M F and Sultan S M. Assessment of genotoxic effect of Escherichia coli in patients with urinary tract infection. Biochem. Cell. Arch. 2021; 21: 2123-2127. DocID: https://connectjournals.com/03

896.2021.21.2123

15. Matthews PC, Maponga T, Ghosh I, Lemoine M, Ocama P, Abubakar I, Story A, Flanagan S. Hepatitis B Virus: Infection, liver disease, carcinogen or syndemic threat? Remodelling the clinical and public health response. PLOS Glob Public Health. 2022; 2;2(12): e0001359. doi:

10.1371/journal.pgph.0001359

. PMID: 36962907; PMCID: PMC10022007.