NTU JOURNAL OF PURE SCIENCES **RESEARCH ARTICLE**
EISSN: 2789-1097 **Open Access**

Evaluation of the genotoxicity and genomic instability in patients with Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Hepatocelular cacinoma (HCC) using micronucleus and comet assay

Alaa Younis Mahdy Alhamadany[1], Wajdi Sabeeh Sadek [2]
Alaa.mahdy156@ntu.edu.iq[1]
1. Northern Technical University, 2. Collage of Sciences, University of Tikrit
Corresponding author: Alaa YounisMahdyALhamadany, e-mail: Alaa.mahdy156@ntu.edu.iq
Received: 17-03-2022, **Accepted:** 13-04-2022, **Published online:** 30-05-2022

Abstract. On somatic cells, the hepatitis B virus (HBV) and the hepatitis C virus (HCV) are mutagenic. HBV and HCV may be causing these mutagenesis effects via integrating into host DNA or by viral proteins. The purpose of this research was to investigate if HBV and HCV had a genotoxic effect on renal epithelial cells' DNA. A total of 145 samples were taken from participants between the periods of 5 \1 2020 and 15\9 2021. (40 healthy controls, HBV 38, 44 HCV, and HCC 23) were used to perform cytogenetic analysis in renal epithelial cells using the micronucleus (MNi) test and comet assay. For the comet experiment, 100 cells were examined for each participant. A total of 100 cells were examined, with MNi scores assigned to each participant. The frequency of MNi was found to be considerably greater in the HBV, HCV, and HCC patient groups than in the control group. There was no significant difference in MNi scores between the HBV, HCV, and HCC patient groups, however there was a significant difference between the study groups and healthy carriers. In conclusion: due to their levels of DNA damage and MNi, chronic HBV, HCV, and HCC patients are afflicted by genomic instability much like other patients.

Keywords: HBV, HCV, HCC, comet assay, Micronucleus test

Introduction

Although other risk factors such as alcohol intake or metabolic liver illnesses can cause liver inflammation, viral infections remain the most common cause of chronic liver disease globally. Hepatitis B, C, and D viruses can cause chronic infection; more than 90% of neonates infected with Hepatitis B Virus (HBV) develop chronic hepatitis, whereas less than 10% of adults do [1]. Chronic hepatitis B and C infections cause 1.3 million fatalities per year due to the rapid advancement of chronic hepatitis to cirrhosis and the development of liver tumors [2].HBV and HCV infections can lead to the development and progression of liver disease, which can range in severity from minor lesions in the parenchyma liver cells to severe fibrosis, cirrhosis, and hepatocellular carcinoma (HCC), which is a major problem associated with HBV and HCV infection[3]. HBV and HCV may promote mutagenic cellular processes such as the integration of the HBV genome into the host DNA and the generation of oxidative stress as a result of the immune response [4]. HBV infects an estimated 35 million people worldwide and is the ninth leading cause of death. Cirrhosis, liver failure necessitating transplantation, and hepatocellular carcinoma (HCC) are only a few of the major clinical implications of HBV infection [5]. HBV and HCV play a complicated and controversial role in liver carcinogenesis via direct and indirect mechanisms. A cis-acting mechanism can activate cellular genes when HBV DNA sequences are integrated into the genome of the host cell. In the micronucleus test (MNi), cytogenetic examination of micronuclei and binucleated cells can be used to determine genotoxicity effects of a substance [6]. During cellular division, MNi is a tiny isolated portion of the nucleus that forms. MNi is formed from chromosomal fragments in interphasic cells. The MNi are cytoplasmic entities that are 20-30% the size of a nucleus and have the same nucleus staining. The frequency of cells containing MNi varies from 0% to 0.9 percent in general. Any chromosomal changes that result in an increase in MNi count. The degree of carcinogenic effect has been linked to the number of MNi [7]. Comet assay, also known as Single-Cell Gel Electrophoresis (SCGE), is another cytogenetic analysis. It is a simple and sensitive technique for assessing genotoxicity by quantifying the amount of damaged DNA caused in individual cells. SCGE is also used to identify damaged DNA in fish, clams, shellfish, and mussels, which is an essential approach for monitoring the health of aquatic creatures[8]. In this investigation, we employed MNi scoring and the comet test to compare the induction of host DNA in the renal epithelial cells of patients infected with HBV, HCV, or HCC to healthy control persons.

Materials and Methods

Sampling

At the periods of 5 1 2020 and 159 2021, 145 samples were taken from subjects (73 females and 72 men). 40 healthy controls, HBV 38, 44 HCV, and HCC 23 are among the samples that have been clinically identified as having symptoms by expert physicians. Their ages range from 16 to 79 years old.

Micronucleus assay in renal epithelial cells

The collection and testing of cytogenetic materials followed biosafety protocols[9]. A midstream clean catch urine sample collects from the second voids of the day was requested from the individuals. The participants were asked to drinking one glass of water every 30 minutes, for three hours before the void, in order to increase the amount of urinary desquamated urothelial cells, also asked, to wash extensively their urethral area thoroughly with tap water and to discharged the first portion of the urine stream into the toilet. Then, the midstream urine sample was collected in sterile containers. These techniques were used to limit the contamination of urine with squamous cells, as well as with the microbial contamination of urine in both males and females. Midstream urine sample was analyzed within two hours. Total volume of voided samples varied from 50 ml and 250 ml; many trials were done to get the best sample volume for both sexes. After collection, urine samples were centrifuged at 2000 rpm for 10 min in centrifuge tube. Then, the supernatant was discarded carefully and resuspended the pellet and transferred to another centrifuge tube. A second centrifugation process was performed at 2000 rpm for 10 min. The supernatant was again discarded, The pellet was washed with 10 ml sterile PBS and centrifuged at 2000 rpm for 10 min. After a second wash, the final pellet was resuspended in 1.0 ml PBS. The final volumes obtained after cell isolation for both sexes samples 0.2 - 1 ml. After that, the slides were fixed with 100 percent methanol and left to air dry before being stained with May Granwald-Gemsa stain.

Scoring

Under a light microscope with a 40x objective magnification, the MNi form was selected and counted according to the procedures previously published by Thomas *et al*. [10]. We counted 100 cells and looked for MNi and nuclear abnormalities.

Evaluation of DNA damage in renal epithelial cells by comet assay

The following steps were used to carry out this test according to Trevigen protocol guidelines. The Lysis Solution was made and then chilled for at least 20 minutes at 4°C. Then, at 37°C, combine cells at 1 x 105 /ml with molten LMA agarose and pipette 50 µl onto CometSlideTM. To achieve total coverage of the sample area, use the side of the pipette tip to disperse agarose/cells across the region. After that, place the slides flat in the refrigerator for 10 minutes at 4°C with the dark field. In high humidity settings, increasing the gelling time to 30 minutes enhances sample adhesion. Then, for 30-60 minutes, immersed slides in 4°C Lysis Solution and drained excess buffer from CometSlideTM.

CometSlideTM was placed in freshly produced Alkaline Unwinding Solution, pH>13, for 1 hour in the dark at 4°C. Place slides in electrophoresis slide tray as slide label near to black cathode and cover with Slide Tray Overlay after adding Alkaline Electrophoresis Solution. The power source was then set to 21 volts and the voltage was applied for 30 minutes. Excess electrophoresis solution was gently drained, and the samples were gently submerged in D.W. twice for 5 minutes each, then in 70% alcohol (Ethanol) for 5 minutes. The samples were dried for 10-15 minutes at 37°C. At this point, samples can be kept at room temperature or scored. The CometSlideTM was stained with 80L 1X Ethidium Bromide, then dipped in cold distilled water to remove the excess stain. The slides are then scored immediately away. The DNA damage was assessed using a fluorescence microscope with a 40X lens and a digital camera to read the slides. The analytic technique was used on four comet photos, with 50 cells picked at random as 25 cells each slide. The quantity of migration per cell, the number of moved cells, and the migration extension between injured cells were all compared.

Viability assay

To establish the quantity of the test material that yields at least 90% viability, a viability experiment should be performed. This was accomplished by combining 10 l of 106 cells/ml in a microcentrifuge tube with 5 µl of trypan blue. After standing for at least two minutes, place on a slide and cover with a cover slip. Score 100 cells and keep track of how many are alive (shiny) and how many are dead (blue).

Statistical analysis

The data was analyzed using a one-way ANOVA. Duncan's unique multiple range test was used to overcome the variation between means as meansSD. For all statistical research, the statistical tool SPSS 26.0 was employed (SPSS Ltd., Surrey, UK).

Results

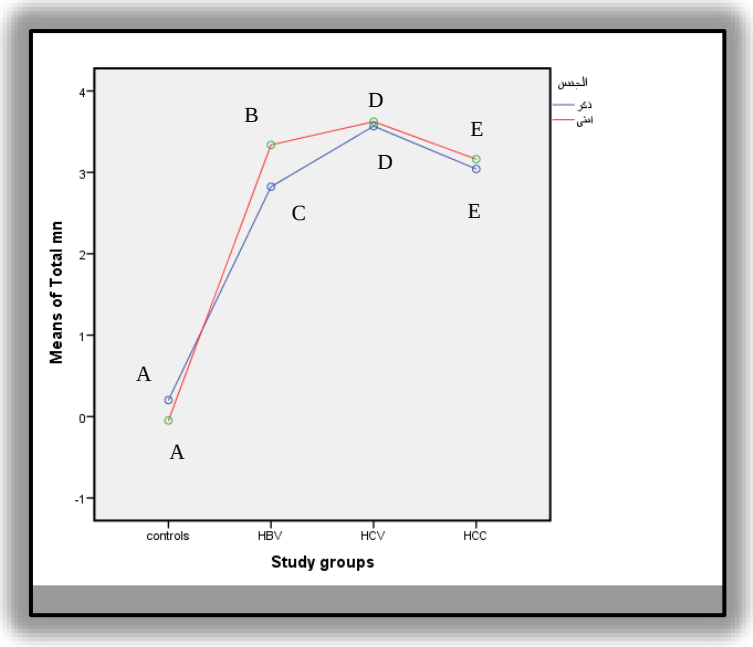
Results of Micronucleus test in renal epithelial cells Table (1) shown the results of mn in renal epithelial cells among groups of HBV patients, HCV patients, and HCC patients groups compared to healthy controls. The results revealed that the highest mn were in patients with HCV (3.77±0.155) followed by HBV patients and HCC patients as (3.47±0.155, 3.04±0.155) respectively, compared to the healthy control group (0.23±0.423). Compare the healthy control group, The results of BN was showed that the HBV patients, HCV patients, and HCC patients groups were lower (0.26±0.049, 0.27±0.049, and 0.22±0.049) respectively than healthy controls group (0.48±0.847). See table (1). with PN, the results showed that the PN in the healthy controls was lower (0.55±0.783) than in the HBV patients' group, and HCV patients' group as (2.92±0.124, 2.89±0.124) respectively whilst it was higher than HCC patient group (0.13±0.124). Same with KR, The results revealed that the KR was lower in the healthy controls (3.53±1.219) than in the HBV patients group, and HCV patients group, (4.11±0.086, 4.09±0.086) respectively whilst it was higher than HCC patient group (3.00±0.086). Furthermore, The results showed that the KL was higher in the healthy controls (1.67±0.944) than in the HBV patients' group (1.50±0.107) while it was lower than HCV patients' group and HCC patients' group (1.95±0.107, 2.61±0.107) respectively. See table(1).

Table 1: Micronucleus assay in oral epithelial cells among study groups

Parameters / *Study groups*		*Total mn*	*BN*	*PN*	*KR*	*KL*	*DIF*
Healthy controls	Mean	0.23	0.48	0.55	3.53	1.67	93.55
	Std. Deviation	0.423	0.847	0.783	1.219	0.944	2.438
	No.	40	40	40	40	40	40
HBV patients	Mean	3.47*	0.26	2.92*	4.11	1.50	87.74
	Std. Error	0.155	0.049	0.124	0.086	0.107	0.305
	No.	38	38	38	38	38	38
HCV patients	Mean	3.77*	0.27	2.89*	4.09	1.95	87.02
	Std. Error	0.155	0.049	0.124	0.086	0.107	0.305
	No.	44	44	44	44	44	44
HCC patients	Mean	3.04*	0.22	0.13	3.00	2.61	91.00
	Std. Error	0.155	0.049	0.124	0.086	0.107	0.305
	No.	23	23	23	23	23	23

*** mean difference is significant at 0.05 level (t-Test), S.E: standard error, Total mn: Total Micronucleus, BN: Binucleated, PN: Pyknotic nucleus, KR:Karyorrhexis, KL:Karyolytic cell, DIF: Normal differentiated cell**

Figure 1. showed the results of total mn in renal epithelial cells among HBV, HCV, and HCC patient groups compared to healthy controls. The results revealed that the total mn in HBV, HCV, and HCC patient groups were higher in females more than males whilst the results of total mn in healthy controls was higher in males more than females (Figure 1).



similar letters indicate that there are no significant differences at the P≤ 0.05 (t-Test).

Figure 1: The main of Total mn in renal epithelial cells in study groups according to gender

Table 2: Total mn in renal epithelial cells in study groups according to age groups

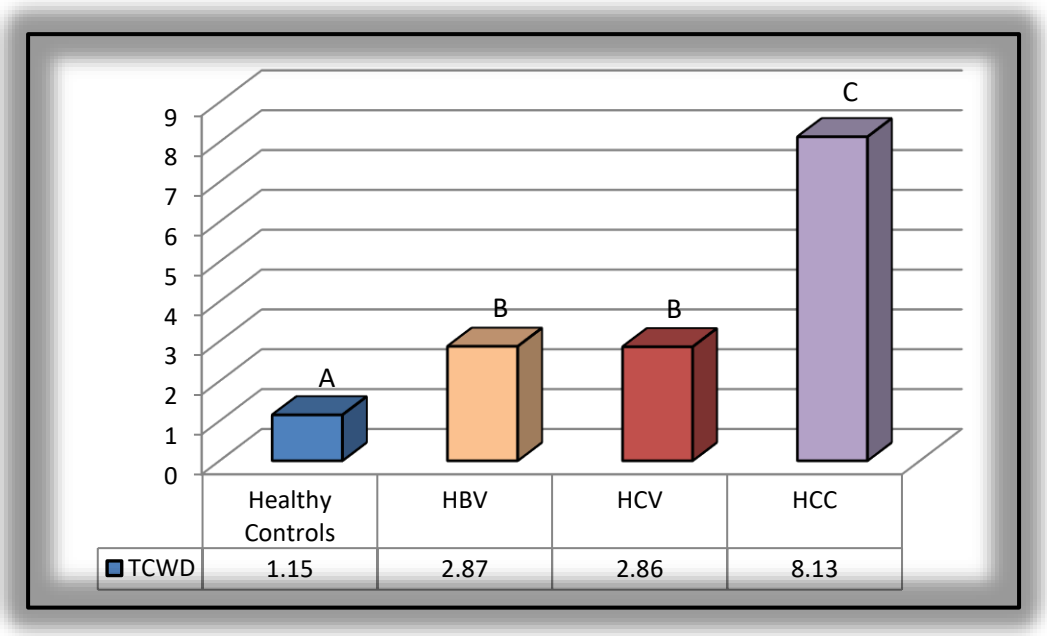
Study groups	**Healthy controls**			**HBV patients**		
Statistical parameters *Age groups (Years)*	Mean	Std. Deviation	No.	Mean	Std. Error	No.
10-20	0.27	0.467	11	-	-	-
20-30	0.11	0.323	18	3.73*	0.263	11
30-40	0.40	0.548	5	3.38*	0.286	13
40-50	0.50	0.707	2	3.50*	0.368	8
50-60	0.33	0.577	3	1.00	0.510	1
60-70	0.00	0.00	1	3.60*	0.510	5
70-80	-	-	-	-	-	-
Total	0.23	0.423	40	3.47*	0.155	38
Study groups	**HCV Patients**			**HCC patients**		
Statistical parameters *Age groups (Years)*	Mean	Std. Error	No.	Mean	Std. Error	No.
10-20	3.50*	0.490	2	-	-	-
20-30	3.69*	0.263	16	-	-	-
30-40	3.81*	0.286	16	2.50*	0.286	4
40-50	4.20*	0.368	5	2.75*	0.368	8
50-60	4.33*	0.510	3	3.00*	0.510	5
60-70	2.50*	0.510	2	4.00*	0.510	4
70-80	-	-	-	3.50*	1.249	2
Total	3.77*	0.155	44	3.04*	0.155	23

The results of mn in renal epithelial cells among HBV, HCV, HCC and healthy controls according to age groups were summarizes in Table 4-4. The results revealed a significantly higher mean of mn in group of HBV patients with age group 20-30 years (3.73±0.263) followed by age group 60-70 years, and 40-50 years (3.60±0.510, 3.50±0.368) respectively. while the lower mean of mn was showed with age group 50-60 years as (1.00±0.510). see Table 2. With HCV patient group, the results showed the highest mean of mn with age 50-60 years was (4.33±0.510) followed by age group 40-50 years, 30-40 years (4.20±0.368, 3.81±0.286) respectively. whilst the lower mean of mn was revealed with age group 60-70 years (2.50±0.510) compared to healthy controls (Table 2). In HCC patient group, the results that showed the mean of mn was highest with age groups (60-70 years (4.00±0.510) followed by age group 70-80 years, 50-60 years (3.50±1.249, 3.00± 0.510) respectively. whilst the lower mean of mn was showed with age group 30-40 years (2.50± 0.286) compared to healthy controls (Table 2). Additionally, the statistical significance of mn test estimated in renal epithelial cells among all groups under study and healthy controls were compatible with clinical significances and showed clinical significances at ($p < 0.05$).

* mean difference is significant at 0.05 level (t-Test), S.E: standard error, Total mn: Total Micronucleus.

Results of Comet assay in oral epithelial cells

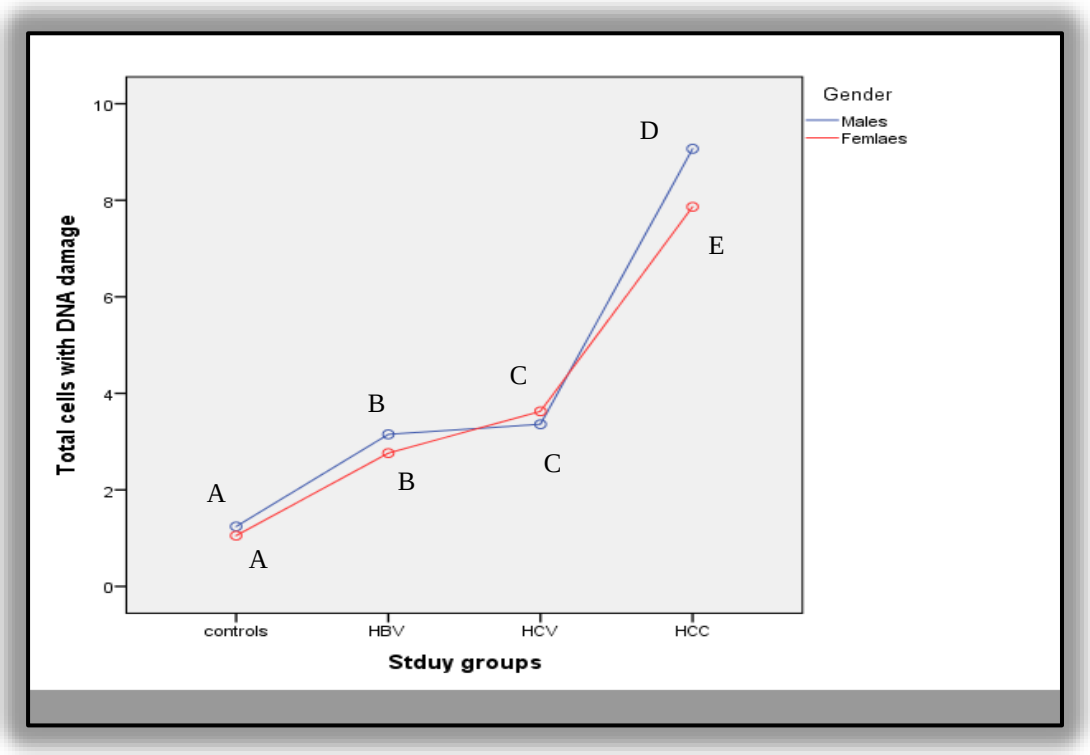
The results of comet assay as for total cells with DNA damage in renal epithelial cells among the study groups were shown in figure (2). The present study revealed that the TCWD was higher in the HCC patient group followed by HBV patient group. While the lower level of TCWD was shown in the HCV patient group compared to the healthy control group (Figure 2). On the other side, the results of the level of total DNA damage in renal epithelial cells were shown in figure (2). the results showed that the level of TD was higher in the HCC patient group followed by the HBV patient group, while the lower level of TD was shown in the HCV patient group compared to healthy controls group (Figure 2).



Different letters indicate that there are significant differences at the P≤ 0.05 (t-Test).
Similar letters indicate that there are none-significant differences at the P≤ 0.05 (t-Test).

Figure 2: Total cells with DNA damage of renal epithelial cells among study groups

The figure (3) shows the results of comet assay as for total cell with DNA damage in renal epithelial cells among HBV, HCV, and HCC patient groups compared to healthy controls according to gender. The results revealed that the TCWD in HBV, and HCC patient groups were higher in males more than females. In contrast , the results of TCWD in HCV patient group were higher in females than in males (Figure 3).



Different letters indicate that there are significant differences at the P≤ 0.05 (t-Test).
Similar letters indicate that there are none-significant differences at the P≤ 0.05 (t-Test).

Figure 3: Total cells with DNA damage of renal epithelial cells among study groups according gender

Table (3) summarizes the results of the comet assay as for total cells with DNA damage in renal epithelial cells among HBV, HCV, HCC, and healthy controls according to age groups. The results showed a significantly higher mean of TCWD in the group of HBV patients with an age group of 60-70 years (4.40 ±0.598) followed by the age groups of 30-40 years, and 50-60 years (3.46±0.336, 3.00±0.598) respectively. while the lower mean of TCWD was shown with the age group of 20-30 years as (2.00±0.309) (Table 3). With the HCV patient group, the present study showed the highest mean of TCWD at age 60-70 years was (5.00±0.598) followed by the age groups 50-60 years, 40-50 years (4.67±0.598; 3.60±0.432) respectively. while the lower mean of TCWD has been shown with age group of 20-30 years (2.31±0.309) compared to healthy controls group (Table 3). Also, the results showed the mean of TCWD in the HCC patient group was higher in the age groups of 70-80 years (11.50±1.464) followed by the age groups 60-70 years and 50-60 years (7.80±0.598; 9.00±0.598) respectively. While the age group of 30-40 years had a lower mean of TCWD (7.25± 0.336) when compared to healthy controls (Table 3). Additionally, the statistical significance of TCWD evaluated in renal epithelial cells among the HBV, HCV, and HCC groups and the healthy controls groups were compatible with clinical significance and showed clinical significance at ($p < 0.05$).

Table 3: Total cells with DNA damage of renal epithelial cells in study groups according to age groups

Study groups	**Healthy controls**			**HBV patients**		
Statistical parameters / *Age groups (Years)*	Mean	Std. Deviation	No.	Mean	Std. Error	No.
10-20	1.36	0.505	11	-	-	-
20-30	1.00	0.767	18	2.00*	0.309	11
30-40	1.20	0.447	5	3.46*	0.336	13
40-50	1.00	0.000	2	2.13*	0.432	8
50-60	1.33	0.577	3	3.00*	0.598	1
60-70	1.00	.	1	4.40*	0.598	5
70-80	-	-	-	-	-	-
Total	1.15	0.622	40	2.87*	0.270	38
Study groups	**HCV Patients**			**HCC patients**		
Statistical parameters / *Age groups (Years)*	Mean	Std. Error	No.	Mean	Std. Error	No.
10-20	2.50*	0.074	2	-	-	-
20-30	2.31*	0.309	16	-	-	-
30-40	2.63*	0.336	16	7.25*	0.336	4
40-50	3.60*	0.432	5	7.50*	0.432	8
50-60	4.67*	0.598	3	7.80*	0.598	5
60-70	5.00*	0.598	2	9.00*	0.598	4
70-80	-	-	-	11.50*	1.464	2
Total	2.86*	0.270	44	8.13*	0.270	23

* Mean difference is significant at 0.05 level (t-Test), S.E: standard error

Discussion

HBV and HCV chronic infections are characterized by highly mutagenic cellular processes such as increased oxidative stress and viral integration into the host cell's DNA [4]. Cell death can be caused by a variety of chemical, physical, and biological causes. There is, however, a class of substances that do not necessarily kill cells, but potentially alter their genetic information. Such factors are referred to as genotoxins. Although many efficient techniques to repair DNA damage have been identified, they occasionally fail, which can lead to damage fixing if the injured cell survives and, if the damaged cell multiplies, to its transmission to subsequent generations. So, the presence of micronuclei (MNi) and nuclear abnormalities (NA) are widely used biomarkers; the detection of MN and NA provides a valuable opportunity to monitor individuals or populations who have been exposed to mutagenic, genotoxic, or teratogenic events, with a focus on the presence of micronucleogenic cells in epithelial tissues such as renal epithelial cells. Furthermore, these indicators can be used to assess chromosomal instability and gene amplification (through nuclear buds), cytokinesis arrest owing to aneuploidy (by binucleated cells), and other cell death events (e.g., karyorrhectic and pyknotic cells). MN detecting may also be used to explain the protective effects against genotoxicity brought on by lifestyle modifications and/or supplement consumption [11]. Some cells may be trapped at a binucleated stage or show nuclear buds (sometimes called "broken eggs" in buccal cells), a biomarker of gene amplification. These indicators of genome damage (e.g., mn, nuclear buds) and cell death (e.g., apoptosis, karyolysis) may be seen in both lymphocytes and buccal cells, providing a more thorough evaluation of genome damage than MN alone in the context of cytotoxicity and cytostatic effects [12].

According to some research, viral hepatitis has mutagenic effects on somatic cells. The viral hepatitis genome can integrate into the host genome in a variety of ways, causing chromosomal instability [13]. These viral DNA integrations are directed not only at the genomes of the host hepatocytes, but also at the genomes of other viruses-carrying cells, such as blood cells. Integrations promote genetic recombination and, in particular, hepatocellular carcinogenesis [14, 15]. HBV infection has been shown in recent research to produce genetic alterations in somatic cells, such as hepatocytes and blood cells, as evidenced by increased chromosomal breakage [13]. These were hepatocyte and cancer cell line studies [16].

MNi frequency in our study was significantly higher in females with HCC than in males, and this finding did not differ from other studies that found MNi frequency was significantly higher in women than men in cancer patients, and this finding did not differ across cancer stages [17]. Many research include the age and gender of the participants, but only a small percentage of these studies were able to find a statistically significant influence by gender or age. Men had a higher mn frequency of buccal cells in two of the studies than women [18, 19]. Humans in today's world are exposed to a variety of genotoxic substances found in the contaminated environment.

Aging is a significant factor in the development of the significant nuclear abnormalities known as micronucleus[20]. The findings of our study indicated that mn frequency increased with increasing age over 50 years among the study groups compared to healthy controls. This finding was acceptable with previous studies, which found that age is an additive factor in increased MN frequency in those above the age of 50 years. The frequency of mn increased with age, increasing in older women above 40 years [21]. An increase in the frequency of chromosomal breakage was linked to higher chromosome instability in the aged of people, however Orta and Günebakan found that the increase in the frequency of micronuclei was initially detected up to 50 years of age, and then decreased with more increasing of age [20]. The frequency of mn and breaking is affected by age, alcohol use, and smoking habits [22]. The age and smoking habits of HBV, HCV, HCC patients, and the control groups are evenly distributed in this study.

The current research showed a relationship between DNA damage, HBV, HCV, and clinically developed HCC. There are just a few clinical experiments that have investigated into whether increased DNA damage in renal epithelial cells is related to chronic viral hepatitis or HCC in patients, and the results are mixed. Although many laboratory findings in HBV transgenic mice show a link between the development of HCC and oxidative DNA damage, human evidence is limited. According to Cardin et al., oxidative DNA damage is correlated to the severity of HCV-related liver cancer [23]. On the other hand, Reshetnyak et al. do not support this hypothesis in chronic HCV or HBV infections[24]. Although DNA damage has been associated to the severity of HCV-related liver disorder, no comparable data exists for HBV-related liver cancer . Notwithstanding the fact that DNA damage has been linked to the severity of HCV-related liver damage, there is no comparable data for HBV-related liver cancer [24].

In the present study, the results showed logical elevation of DNA damage with increasing the severity of viral hepatitis infection. These results were comparable with previous studies. Given that HCV infection both cause DNA damage, it is reasonable to predict that HCV subjects will have considerably more DNA damage than non HCV subjects [25, 26]. As a result, it's suggests that a lower HCV viral load is one of the mechanisms limiting future increases in DNA damage in HCV patients. As a result, no evidence indicating an extra increase in DNA damage among HCV subjects has been found, confirming our findings. Despite the fact that the incidence of liver cancer among HCV subjects has been reported to be 4.3 %, which is significantly higher than the incidence of liver cancer among non HCV subjects, it appears to be comparable to the reported incidence of liver cancer among HCV subjects without renal disease [27].

In the present study, the incidences of DNA single-strand breaks by comet assay in renal epithelial cells from patients with B and C viruses and from patients with HCC related to was examined. Bio-monitoring and nutritional research investigating at DNA damage would benefit greatly from an epithelial-based comet assay cell model employing cells that can be obtained readily and safely from human participants. Buccal cells have been employed in three comet test results that have been published thus date [28]. Two research have demonstrated the existence of substantial DNA damage in epithelial cells of people with chronic HCV infection, as measured by the 8-hydroxyguanosine content, and showed that it is associated with that of liver tissue and greater than in HBV patients [23]. In a previous research [29], DNA damage levels considerably greater than in healthy individuals were discovered in epithelial cells of patients with chronic hepatitis C and HBV-related cirrhosis using the alkaline comet test. In a fourth research, however, any increase in the frequency of DNA single-strand breaks and alkali labile sites in cells from chronic hepatitis B or C patients was shown to be missing [30].

Gender, age, and nutrition all impact Comet test responses in lymphocytes when assessing human genotoxicity [31]. Males in a healthy Indian population had greater levels of baseline DNA damage than females, according to a prior study [31]. Smoking, a non-vegetarian diet, and age all contributed to an increase in DNA damage in males [32]. Males have a greater amount of DNA damage in lymphocytes than females, according to studies in Italian [33]and Chinese populations [34], and smoking is linked to increased Comet assay responses. Increased DNA damage in lymphocytes is also linked to aging [32].

The increased levels of DNA damage in HCC hint to the possibility of using DNA damage for therapeutic applications in cancer. Because there are no effective curative therapies for HCC, it is critical to avoid hepatocarcinogenesis at an early stage. Antiviral treatment may be able to largely eradicate HCV's cancer-promoting effects. Regrettably, clinical evidence indicates that treatment effectiveness is still limited. Novel therapies for avoiding DNA damage and enhancing the biological capacity for DNA damage repair might open up new options for prevention.

Acknowledgments. The authors would like to thank the departments of Northern Technical University's Technical Institute Mosul for allowing them to perform their research at their lab.

References

1. Trépo, C., Chan, H.L.Y. and Lok A. (2014). Hepatitis B virus infection. Lancet. 384:2053–2063.
2. World Health Organization . (2017). Global Hepatitis Report, 2017. WHO; Geneva, Switzerland.
3. Szabó E, Páska C, Kaposi Novák P, Schaff Z, Kiss A. Similarities and differences in hepatitis B and C virus induced hepatocarcinogenesis. Pathol Oncol Res. 2004;10:5–11.
4. Sung, WK., Zheng, H., Li, S., Chen, R., Liu, X., Li ,Y., Lee, NP., Lee, WH., Ariyaratne, PN., Tennakoon, C., Mulawadi ,FH., Wong, KF., Liu, AM., Poon, RT., Fan, ST., Chan, KL., Gong, Z., Hu, Y., Lin, Z., Wang, G., Zhang, Q., Barber, TD., Chou, WC., Aggarwal, A., Hao, K., Zhou, W., Zhang, C., Hardwick, J., Buser,C., Xu, J., Kan, Z., Dai, H., Mao, M., Reinhard, C., Wang, J. and Luk ,JM. (2012). Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. Nat Genet. 44:765–769.
5. LopezV, BourneE, LutzM, Condreay L. Assessment of the COBAS amplicor HBV monitor test for quantitation of serum hepatitis B virus DNA levels. J. Clin. Microbiol. 2002; 40: 1972--1976.
6. SurrallesJ, and Natarajan AT. Human lymphocytes micronucleus assay in Europe. An international survey. Mutat. Res. 1997; 392, 165--174.
7. Nezhad M D, Naderi N J, Semyari H. Micronucleus Assay of Buccal Mucosa Cells in Waterpipe (Hookah) Smokers: A Cytologic Study. Iran J Pathol. 2020; 15(2): 75-80.
8. Tasneem, S and Yasmeen R . Evaluation of genotoxicity by comet assay (single-cell gel electrophoresis) in tissues of the fish Cyprinus carpio during sub-lethal exposure to Karanjin. The Journal of Basic and Applied Zoology. 2018; 79:19.
9. Koss, LG. and Melamed, MR .(2005). The lower urinary tract in the absence of cancer, in Koss, L.G., Melamed, M.R. (Eds.), Koss' Diagnostic Cytology and its Histopathologic Bases, 5th ed. Lippincott Williams & Wilkins, Philadelphia . pp. 738-771.
10. Thomas, P., Holland, N., Bolognesi, C., Kirsch-Volders, M., Bonassi ,S., Zeiger, E., et al. (2009). Buccal micronucleus cytome assay. Nat Protoc. 4:825-37.
11. Torres-Bugarin, O., Zavala-Cerna, M. G., Flores-Garcia, A. and M. Ramos-Ibarra, L. (2013). Procedimientos básicos de la prueba de micronúcleos y anormalidades nucleares en células exfoliadas de mucosa oral," El Residente. 1:4–11.
12. Speit, G. and Schmid, O. (2006). Local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated epithelial cells. Mutat Res. 613:1-9.
13. Huang , JM., Huang, TH., Qiu, HY., Fang, XW., Zhuang, TG., Liu, HX., Wang, YH., Deng, LZ. and Qiu, JW. (2003). Effects of hepatitis B virus infection on human sperm chromosomes. World J Gastroenterol. 9(4): 736-740.
14. Chatterjee,B. and Ghosh,P.K. (1989) Constructive heterochromatin polymorphism and chromosome damage in viral hepatitis. Mutat. Res., 210, 49--57.
15. Zondervan,P.E., Wink,J., Alers,J.C., Ijzermans,J.N., Schalm,S.W., De Man,R.A. and Van Dekken,H. (2000). Molecular cytogenetic evaluation of virus-associated and non-viral hepatocellular carcinoma: analysis of 26 carcinomas and 12 concurrent dysplasias. J. Pathol. 192, 207--215.

16. Livezey,K.W., Negorev,D. and Simon,D. (2002) Increased chromosomal alterations and micronuclei formation in human hepatoma HepG2 cells transfected with the hepatitis B virus HBX gene. Mutat. Res., 505, 63--74.

17. Ambroise, MM., Kanchana, B. and Manjiri , P. (2013). Predictive value of micronucleus count in cervical intra epithelial neoplasia and carcinoma. Turk Patolji Derg. 29:171–78.

18. Pastor, S., Gutierrez, S., Creus, A., Cebulska-Wasilewska, A. and Marcos, R. (2001). Micronuclei in peripheral blood lymphocytes and buccal epithelial cells of Polish farmers exposed to pesticides. Mutat Res. 495:147- 56.

19. Gattas, GJ., Cardoso Lde , A., Medrado-Faria Mde, A. and Saldanha, PH. (2001). Frequency of oral mucosa micronuclei in gas station operators after introducing methanol. Occup Med (Lond). 51:107-13.

20. 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.

21. Misra, JS., Srivastava, S., Singh, U. and Srivastava, AN. (2009). Risk-factors and strategies for control of carcinoma cervix in India: Hospital based cytological screening experience of 35 years. Indian Journal of Cancer. 46(2):155–59.

22. Channarayappa,J.N. and Ong,T. (1990). Micronuclei assay in cytokinesisblocked binucleated and conventional mononucleated methods in human peripheral lymphocytes. Teratog. Carcinog. Mutagen., 10, 273--279.

23. Cardin, R., Saccoccio, G., Masutti, F., Bellentani, S., Farinati, F. and Tiribelli, C. (2001). DNA oxidative damage in leukocytes correlates with the severity of HCV-related liver disease: Validation in an open population study. J. Hepatol. 34: 587–92.

24. Reshetnyak, VI., Sharafanova, TI., Ilchenko, LY. and Poroshenko, GG. (2002). DNA structure in peripheral blood lymphocytes from patients with chronic viral liver damages. Bull. Exp. Biol. Med. 133: 399–400.

25. Kan E., Undeger U., Bali M., Basaran N., Assessment of DNA strand breakage by the alkaline COMET assay in dialysis patients and the role of Vitamin E supplementation, Mutat. Res. 520 (2002) 151–159.

26. Stopper, H., Boullay, F., Heidland, A., Vienken, J. and Bahner U. (2001). Comet assay analysis identifies genomic damage in lymphocyles of uremic patients, Am. J. Kidney Dis. 38:296–301.

27. Nakayama, E., Akiba T., Marumo, F. and Sato, C. (2000). Prognosis of antihepatitis C virus antibody-positive patients on regular hemodialysis therapy, J. Am. Soc. Nephrol. 11 : 1896–1902.

28. Eren, K., Ozmeriç, N. and Sardaş, S. (2002). Monitoring of buccal epithelial cells by alkaline comet assay (single cell gel electrophoresis technique) in cytogenetic evaluation of chlorhexidine. Clin Oral Investig. 6(3):150-4.

29. Bolukbas, C., Bolukbas, FF., Kocygit ,A., Asian, M., Selek, S., Bitiren, M. and Ulukanligil, M. (2006). Relationship between levels of DNA damage in lymphocytes and histopathological severity of chronic hepatitis C and various forms of hepatitis B. J Gastroenterol Hepatol. 21:610–616.

30. Reshetnyak, VI., Sharafanova, TI., Ilchenko, LU., Golovanova, EV. And Poroshenko, GG. (2001). Peripheral blood lymphocytes DNA in patients with chronic liver diseases. World J Gastroenterol .7:235–237.

31. Bajpayee, M., Dhawan, A., Parmar, D., Pandey, A.K., Mathur, N. and Seth, P.K. (2002). Gender related differences in basal DNA damage in lymphocytes of a healthy Indian population using alkaline Comet assay, Mutat. Res. 520:83–91.

32. Piperakis,S.M., Visvardis, E.E., Sagnou, M. and Tassiou, A.M. (1998). Effects of smoking and aging on oxidative DNA damage of human lymphocytes, Carcinogenesis. 19 : 695–698.

33. Lam, T.H., Zhu, C.Q. and Jiang, C.Q. (2002). Lymphocyte DNA damage in elevator manufacturing workers in Guangzhou, China, Mutat. Res. 515 :147–157.