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Analysis of the Chromosomal Aberrations in Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and Hepatocellular Carcinoma (HCC)Patients in Mosul City, Iraq

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Abstract. Hepatocellular carcinoma (HCC) (also known as liver cancer) is one of the most frequent cancers in humans. HCC is linked to chronic hepatitis B and C virus infection, cirrhosis, and excessive alcohol consumption. The aim of this study was to use Metaphase chromosome analysis in whole blood to determine chromosomal aberrations (CA) in HBV, HCV, and HCC patients. A cohort of 145 samples have been collected from participants from the date of 5 \ 1 \ 2020 to 15\9\ 2021. Among those samples are (40 healthy controls, HBV 38, 44 HCV, and HCC 23) to make cytogenetic evaluation by observing the analysis of chromosome aberration. Our study showed that the chromosome aberration With Gap was higher in the HCC patient group followed by HBV patient group. also, the results showed that of the chromosome aberration without Gap in the HCC, and HCV patient groups were significantly higher in females than in males. The results showed that higher break, dicentric (DIC) was in HBV patients group followed by HCV patients group. The results showed that deletion, and ace were higher in the HCV and HCC patient group followed by HBV patient group. Finally with Robertsonian translocations(RO. Trans), the results showed that ace was higher in the HCV, and HBV patient groups. In conclusion, we indicate that HBV, HCV, and HCC patients have chromosomal instability because of their chromosome aberration levels.

Keywords: chromosomal aberrations, HBV, HCV, Karyotype

Introduction Karyotypic research has revealed essential knowledge about chromosomal aberrations (CA) in a variety of cancers. Furthermore, CA in peripheral blood cell culture is a characteristic of solid tumors, and chromosomal rearrangements have been recognized for decades to exist in most, if not all, human tumors [1]. Chromosome material deletions are prevalent and have a non-random pattern in HCC, with recurrent deletions on chromosomes 1p, 4q, 8p, 13q, 16q, and 17p [2]. The association between an increased frequency of CA and the presence of hepatitis B virus (HBV) in the blood, as well as the fact that chronic carriers with detectable HBV in the blood had the greatest frequency of chromosomal breaks, imply that HBV may be involved in these genomic lesions [3]. According to Chatterjee and Gosh [4], patients in the acute phase of hepatitis A and B virus infection had an increased amount of CA and sister chromatid exchanges in their metaphase chromosomal spreads.

Today, conventional chromosome karyotype analysis enables the researchers to detect minute chromosome changes like chromosomal deletions, duplications, translocations, or inversions. The amalgamation of medical genetics with clinical medicine serves as a source of diagnostic information for different genetic disorders, birth defects, and cancers. So, it is very important to have enhanced and optimized the karyotype protocols [5]. The assembled karyotype is then interpreted and identified for any changes in structures, aneuploidy, and the presence of unknown genetic material in chromosomes. This methodology has been a helps in diagnostic subtyping, prognosis, and monitoring of diseases of cancer [6]. On the other hand, the karyotype has disadvantages that rely on living cells to culture, and harvest of viable cells may be limited by exposure of chemotherapy. Also, slightly genetic rearrangements can't be detected. In this study, we analysis the chromosomal aberration and genomic instability of host DNA infected with HBV or HCV and HCC patients compared to healthy control individuals. To our knowledge, this is the first study of its novel type in HBV, HCV, and HCC patients in Mosul City, Iraq.

Materials and Methods

Sampling

A cohort of 122 individuals have been participants in this study (64 females and 58 males) from the date of 15\ 1 \ 2020 to 15\ 9 \ 2020. Among those samples are (38 HBV, 44 HCV, and 40 healthy controls) they have been clinically diagnosed that may have signs by specialized physician's and laboratory tests. Their ages are ranging from 16- 70 years.

Chromosomal Analysis

The chromosomal aberration was analyzed using 5 ml of heparinized peripheral blood samples for karyotyping. Standard cytogenetic procedures were used to produce metaphase spreads on phytohaemagglutinin (PHA) activated peripheral cells [7]. In chromosomal medium, lymphocytes were developed (5 ml). PHA was used as a mitotic stimulant (0.5 ml inoculum), and the samples were cultured in a 37°C incubator for 72 hours. With the addition of 150µl of 0.1% colchicine, the cells were arrested in metapha

Harvesti After 72 hours, the cultures were collected, centrifuged for 10 minutes at 1500 rpm, and the supernatant was discarded. The cultures were hypotonic treated with KCl (5to 10 ml), centrifuged a second time at 1500 rpm for 10 minutes, and the cells were fixed with three changes of fixative (3:1, methanol:acetic acid). The fixative washes were repeated two to three times until a clear suspension was produced. Giemsa stain was used to stain the prepared slides.

Table (1): structural Chromosome aberrations in peripheral whole blood among study groups

Parameters / Study groups		Structural Aberration			
		chromosome aberration With Gap	chromosome aberration Without Gap	chromosome aberration With Gap	chromosome aberration Without Gap
		Mean ± Std. Deviation		Mean ± Std. Error	
Healthy controls	Males	0.62±0.650	0.38±0.650	-	-
	Females	0.41±0.572	0.33±0.555	-	-
	Total	0.48±0.599	0.35±0.580	-	-
HBV	Males	-	-	1.28±0.093*	1.00±0.068*
	Females	-	-	1.25±0.093*	0.85±0.068*
	Total	-	-	1.26±0.093*	0.92±0.068*
HCV	Males	-	-	1.04±0.093*	0.74±0.068*
	Females	-	-	1.41±0.093*	0.88±0.068*
	Total	-	-	1.18±0.093*	0.80±0.068*
HCC	Males	-	-	1.43±0.093*	1.07±0.068*
	Females	-	-	2.33±0.093*	1.78±0.068*
	Total	-	-	1.78±0.093*	1.35±0.068*

Statistical analysis

Using one-way ANOVA, the data was analyzed. To resolve the variance between treatment means, Duncan's novel multiple range test was applied. The statistical program SPSS 28.0 was used for all statistical analyses (SPSS Ltd., Surrey, UK)

Results

Table (1) has been shown the results of chromosomes aberration on peripheral blood among HBV, HCV, HCC, and healthy controls groups. Our study showed that the chromosome aberration With Gap was higher in the HCC patient group followed by HBV patient group as (1.78±0.093, 1.26±0.093) respectively . While the chromosome aberration With Gap in the HCV patient group was shown as 1.18±0.093 compared to the healthy control group as 0.48±0.599. Similarity, the results of the chromosome aberration without Gap in peripheral whole blood culture has been shown higher in the HCC patient group followed by the HBV patient group (1.35±0.068, 0.92±0.068) respectively , while the chromosome aberration without Gap lower in the HCV patient group was shown as 0.80±0.068 compared to healthy controls group 0.35±0.580 (Table 1). Furthermore, Table 1 shown the results of chromosome aberration With Gap in peripheral whole blood culture among study groups compared to healthy controls group according to gender. The results of our study revealed that the chromosome aberration With Gap in HCC, and HCV patient groups were higher in females (2.33±0.093, 1.41±0.093) respectively more than males(1.43±0.093, 1.04±0.093) respectively. In contrast , the results of chromosome aberration With Gap in HBV patient group were higher in males 1.28±0.093 than in females 1.25±0.093 (Table 1). Similarity, The present study showed that of the chromosome aberration without Gap in peripheral whole blood culture in the HCC, and HCV patient groups were significantly higher in females (1.78±0.068, 0.88±0.068) respectively than in males (1.07±0.068, 0.74±0.068) respectively. In contrast, the result showed slightly higher in males 1.00±0.068 than in females 0.85±0.068 in the HBV patient group compared to healthy controls group compared to healthy controls group (Table 1). Moreover, table 1 shown the results of chromosome aberration With Gap in peripheral whole blood culture among study groups compared to healthy controls group according to gender. The results of our study revealed that the chromosome aberration With Gap in HCC, and HCV patient groups were higher in females (2.33±0.093, 1.41±0.093) respectively more than males(1.43±0.093, 1.04±0.093) respectively. In contrast , the results of chromosome aberration With Gap in HBV patient group were higher in males 1.28±0.093 than in females 1.25±0.093 (Table 1). Similarity, The present study showed that of the chromosome aberration without Gap in peripheral whole blood culture in the HCC, and HCV patient groups were significantly higher in females (1.78±0.068, 0.88±0.068) respectively than in males (1.07±0.068, 0.74±0.068) respectively. In contrast, the result showed slightly higher in males 1.00±0.068 than in females 0.85±0.068 in the HBV patient group compared to healthy controls group compared to healthy controls group (Table 1).

Also, the results of structural chromosome aberration (Gap, Break, Deletion, DIC, Ace, and RO. Trans.) were shown in table (2). The results showed that Gap was higher in the HCV patient group followed by HBV patient group as (37.77 %, 28.88%) respectively . While the HCC patient group was shown as 22.22% compared to the healthy control group as 11.11 %. With break, the results showed that higher break was in HBV patients group followed by HCV patients group as (32.75%, 27.58%)respectively. while HCC patients group was shown as 25.86% compared to healthy group (13.79%). With Deletion, the results showed that deletion was higher in the HCV and HCC patient group followed by HBV patient group as (35 %, 35%, 25) respectively compared to the healthy control group as 5 %. With DIC, the results showed that DIC was higher in the HBV, and HCV patient group followed by HCC patient group as (30 %, 30%, 25) respectively compared to the healthy control group as 15 %. With ace, the results showed that ace was higher in the HCV, and HCC patient group followed by HBV patient group as (33.3 %, 33.3%, 22.2) respectively compared to the healthy control group as 11.1 %. Finally with RO. Trans., the results showed that ace was higher in the HCV, and HBV patient groups as (37.5%, 37.5%) respectively. while the HCC patient group showed the same results of the healthy control group as 12.5 % each one (Table 2). On other side, Table (2) has been also shown the results of numerical aberration of chromosomes among HBV, HCV, HCC, and healthy controls groups. The present study showed that the aneuploidy numerical aberration of chromosome was higher in the HBV patient group followed by HCV patient group as (40%, 30%) respectively . While the HCC patient group was shown as 20% compared to the healthy control group as 6%. Also, the study revealed the same results of polyploidy numerical aberration of chromosome was in the HBV,

HCV, and HCC patient groups as 33.3%. while the polyploidy numerical aberration of chromosome in healthy controls group not found (Table 2).

Table (2): Chromosome aberrations(Gap, Break, Deletion, DIC, Ace, and RO. Trans) in peripheral whole blood among study groups

		Structural Aberration						Numerical Aberration	
Parameters / Study groups		Gap	Break	Deletion	DIC	Ace	RO. Trans.	Aneuploidy	Polyploidy
		%						%	
Healthy controls	Males	6.66	3.44	-	10	11.11	-	2	-
	Females	4.44	10.35	5	5	-	12.5	4	-
	Total	11.11	13.79	5	15	11.11	12.5	6	-
HBV	Males	11.11	15.51	20	15	-	25	16	33.3
	Females	17.77	17.24	5	15	22.22	12.5	24	-
	Total	28.88	32.75	25	30	22.22	37.5	40	33.3
HCV	Males	17.77	15.51	30	5	33.33	12.5	16	-
	Females	20	12.07	5	25	-	25	14	33.3
	Total	37.77	27.58	35	30	33.33	37.5	30	33.3
HCC	Males	11.11	10.35	15	15	22.22	12.5	10	33.3
	Females	11.11	15.51	20	10	11.11	-	14	-
	Total	22.22	25.86	35	25	33.33	12.5	24	33.3

Discussion

The current study showed that the structural of chromosomal aberrations was greatly elevated after the infection of hepatitis B virus, and FISH could directly visualize the integration of HBV DNA sequences into sperm chromosomes. These results suggested that HBV infection could produce inheritable biological effects by carrying genetic materials damaged by virus or carrying altered genetic constituent due to the insertions of virus DNA in germ cells. As we know, this is the first report about the influence of biological factors on human sperm chromosomes. In HCC, HBV, and HCV, total chromosomal aberrations with gaps were shown to be significantly higher. These findings revealed that HBV and HCV infection potentially cause inheritable biological consequences by inducing damage to genetic materials or altering genetic constituents owing to viral DNA insertions in cells [8]. The etiology of chromosomal aberrations in HBV and HCV infected patients are unclear. The following are some of the possibilities we considered: First, antigen components of viral hepatitis, such as core protein, may interfere with the assembly of chromosome from chromatin due to its interaction with histones; it was confirmed that the core protein had participated in the organization of nucleosomes with histones in the HBV, and HCV minichromosomes [9], so the condensation degree of chromosome was reduced and chromosome staining became more difficult. Second, virus or its components caused local despiralizations in the chromosomes. Third, HBV and HCV may cause premature chromosomal condensation (PCC) since virus-induced PCC is a typical occurrence [8].HBV and HCV infections have been shown to cause genetic changes in somatic cells such as hepatocytes and blood cells, with a rise in the rate of chromosomal aberrations or SCEs[10]. The incidence of chromosomal aberrations in the HBV, HCV, and HCC study groups was significantly higher than in healthy controls, according to our results. HCC tissue revealed a number of deletions, inversions, tandem duplications, and translocations. Copy number variations and other genetic aberrations can be caused by

chromosomal rearrangements, which could serve as an early noninvasive diagnostic for HCC[11]. In a wide spectrum of malignant tumors, karyotypic analysis has offered useful information on chromosomal aberrations (CA). Furthermore, CA in peripheral blood cell culture is a characteristic of solid tumors, and chromosomal rearrangements have been known for decades in most, if not all, human tumors [1].We found that chromatid aberrations (CA) were prevalent in both experimental and control participants in the present study. In groups of HBV, HCV, and HCC patients, the experimental participants had a higher number of chromatid type aberrations (Table 1). The majority of HCC patients exhibited very complicated chromosomal alterations, which resulted in incomplete karyotypes in most cases. In human HCC, cytogenetic investigations have indicated common abnormalities in chromosome 1q, such as translocation, trisomy, or amplification [12], as well as loss of heterozygozity in the chromosomal regions 1q42–43 and 2q35–37 [13]. Data from previous study, comparative genomic hybridization (CGH) revealed frequent gains of chromosomal areas 1q (57.1%), 8q (46.6%), 6p (22.3%), and 17q (22.2%), as well as notable losses of 8p (38%), 16q (35.9%), 4q (34.3%), 17p (32.1%), and 13q (26.2%) [12]. In HBV-related malignancies, chromosomal losses in the areas 4q, 13q, 16q, and 8p are more common, although loss of chromosome 8p in HCV-positive HCCs is less common compared to virus-negative tumors [15]. Furthermore, HCC progression has been demonstrated to enhance gains of 1q and 8q, as well as losses of 4q, 16q, and 13q [15]. Gaps, breaks, deletions, and dicentrics were all prominent CA in this investigation. Chromosome deletion is a common CA in HCC that has been identified in several other studies [16]. Chromosome deletions are common in HCC and occur in a non-random pattern, with deletions on chromosomes 1p, 4q, 8p, 13q, 16q, and 17p [17]. The most typically reported areas of interest in other clinical investigations include the 8p deletion. In one investigation, HBV-related HCC impacted chromosomes 4q, 8p, and 16q, while HCV-related HCC damaged chromosome 11q [18]. In addition, HBV-HCV-related CA was often seen in 5p and 8p in a research. Human HCC has been found to have frequent allelic deletion in various chromosomal locations, including 1p, 4q, 6p, 8p, 13q, 16q, and 17p[19]. Our findings show that the deletion of 8p is linked to the development of HCC due to the high frequency of the deletion.

Another possibility for HCV-induced hepatocarcinogenesis is that HCV has a direct carcinogenic effect. Inappropriate expression of two growth factors linked to hepatic carcinogenesis, transforming growth factor- and insulin-like growth factor II, may be caused by viral replication[20]. NS3, a non-structural HCV protein, can serve as a protease and a helicase. As a result of its helicase activity, HCV may cause genomic instability and encourage mutations. The protein's function is comparable to that of protein kinase A, and it has the potential to disrupt cellular homeostasis [21]. In conclusion, the current investigation found that lymphocytes from patients with HCC, and patients infected with HBV or HCV had more chromosomal instability, as evidenced by the occurrence of chromosomal aberration such as gaps, breaks, deletion, and DIC.

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