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Article

THE ASSOCIATION OF DNA REPAIR GENE XRCC3 T241M POLYMORPHISM AND THE RISK OF HCV INDUCED HCC IN A COHORT FROM THE EGYPTIAN POPULATION

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ABSTRACT: Background: Approximately 10-15% of the Nile Delta population are suffering from hepatitis C virus (HCV) epidemic. A genomic analysis has revealed the potential role of genetic variants as important determinants of susceptibility to hepatocellular carcinoma (HCC). Egyptian cirrhotic patients have a high rate of HCC. The relationship between *XRCC3* polymorphism and HCC risk has been the focus of many researches. Unfortunately, they have reached conflicting conclusions. The goal of the current study was to clarifying the correlation between *XRCC3* T241M gene polymorphism and risk of development of HCC in HCV-infected Egyptian patients and to explore the role that polymorphism plays in the transition from chronic liver injury to dysplasia and ultimately HCC. A total of one hundred sixty-five (n=165) subjects were inscribed in this study and were subdivided into three groups. Group I included 50 healthy individuals who served as a control group. Group II included fifty-five patients (n=55) diagnosed with HCV, and Group III included sixty patients (n=60) with an established diagnosis of HCC in addition to HCV. **Results:** The results of the present study showed that the prevalence of *T allele* was highly frequent among HCC group (0.617) compared to the HCV group (0.545) and the control group (0.39). On the other hand, the *C allele* was remarkably frequent among the control group (0.61) compared to the HCV group (0.455) and the HCC group (0.383). There was a highly significant difference between the HCV and HCC groups in the allele frequencies relative to the control group (p=0.001). However, there was no substantial difference in allele frequencies between the HCV and HCC groups (p=0.08). Similarly, the *TT genotype* was highly frequent among the HCC group (45%) and the HCV group (29.1%) compared to the control group (12%). Moreover, the *CC* and *CT genotypes* were higher among the control group than both the HCV and HCC groups. **Conclusions:** The data obtained from the current study suggest that *XRCC3 CT* (rs861539) mutation may contribute to HCC development. Moreover, *XRCC3 CT* (rs861539) shows a strong correlation with HCC susceptibility in a high-risk (HCV-infected) Egyptian population.

Key words: *XRCC3* T241M, polymorphism, HCV, HCC, Egypt.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most popular ones of malignancy. It was the sixth most common form of cancer and is considered the fourth leading cause of death associated with cancer worldwide. **[1]**. The geographical prevalence of HCC is connected to chronic hepatitis C virus (HCV) infection due to high HCV prevalence that is strongly predisposes to development of HCC. In Egypt, the proportion of HCC among cirrhotic patients was markedly increased. This increasing incidence may be explained by high prevalence of HCV in Egypt. Unfortunately, Egypt has world's highest prevalence of HCV and it represents 63% of diagnosed HCC cases **[2]**. Approximately 10-15% of the Nile Delta population were suffered from HCV over the past 3 decades **[3]**.

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Hepatocellular carcinoma occurs in a number of conditions, which lead to long term damage and scarring of the liver cirrhosis that may be caused by chronic viral hepatitis (B &C) and non-viral causes such as alcoholic, non-alcoholic cirrhosis, and autoimmune liver diseases **[4]**. The possible contribution of genetic variants to HCC susceptibility was emphasized through a genome-wide association study. Many studies in molecular epidemiology displayed that accumulation of genetic factors and epigenetic abnormalities may also play a role in carcinogenesis of the liver **[5]**.

One of the primary causes for liver cirrhosis and HCC is viral hepatitis. It is estimated to be the 7th leading cause of mortality wold wide. HCV is responsible for half of this mortality. The Egyptian Demographic and Health Surveys (EDHS) conducted more than one survey to test serological evidence of past or current infection for age-specific prevalence. They elucidated decrease in incidence over time **[6]**.

In spite of the significant advances in genetic epidemiology, unveiling the genetic architecture of HCC remains a demanding task. The true effect of the culprit genotype obscured by conflicting data obtained from underpowered studies and make it difficult to draw solid conclusion **[7]**. Novel genetic biomarkers in HCV infection that could contribute to HCC susceptibility have explored by several studies. Clear awareness of the genetic effects of viral infection on clinical course is of great clinical interest to enhance the current guidelines for genetics-based patient screening which in turn will used to improve preventative, diagnostic and therapeutic strategy. Consequently, improving the use of recently developed but highly costly direct-acting antiviral (DAA) therapy **[8]**. The identification of such genetic risk markers can provide proof to explore the pathogenesis of insights and to develop new therapeutic strategies for HCC

The fundamental pathognomonic event of cancer is increased genetic instability as outcome of DNA damage and reduce DNA repair capacity (DRC). The DNA damage response (DDR) genes account for inter-individual variation in DRC and defects in DDR genes are related to susceptibility of cancer. The base excision repair (BER) could especially eliminate methylation among various DNA repair pathways, oxidation or alteration of a single base that further restores single-strand DNA breaks whereas the homologous recombination repair (HRR) could restore DNA double-strand DNA breaks. It is challenging to move from selecting DDR gene candidates to enumerate the casual variants. It is a useful shortcut to challenge a non-synonymous variant of coding with good biological characteristics within a persuasive gene **[6]**.

The cross-complementing group 3 (XRCC3) X-ray repair gene is located on chromosome *14q32.33 and* is involved in the BER pathway through repairing DNA single-strand breaks. The *XRCC3* T241M polymorphism (rs861539) comprises of a *CT* transition that result in a substitution of amino acid at codon 241 from threonine to methionine. For this polymorphism, three genotypes were identified: wild-type (*CC*), heterozygote (*CT*) and homozygote (*TT*). *Variation of the XRCC3 sequence* may affect the encoded protein function and thereby reduce its DRC. The *XRCC3 CT* polymorphism increases the risk of HCC and other malignancies such as lung cancer and cervical cancer **[9]**, and also likely to play a significant role in HCC prognosis **[1]**.

The association between *XRCC3* polymorphism and hepatocarcinogenesis have been investigated in many previous studies but unfortunately, their results were inconclusive. Incoherence may be due to heterogeneity factors, including limited samples and various ethnic groups. This relevant study aimed to assess the correlation between the *XRCC3 CT* gene polymorphism and HCC risk in patients of HCV in Egypt and to identify its new role as a predictive genetic marker or as a therapeutic target to treat patients with HCC in addition to HCV.

METHODS

Study population and demographic information

This research was carried out at the authors' institution. One hundred and sixty-five participants were inscribed in the study and divided into 3 groups. Group I involved 50 healthy individuals who served as a control group (36 males/ 14 females) and whose age varied between 32 and 57 years (mean ± SD = 46.7±13.3). Group II included 55 patients diagnosed with HCV (27 males/ 28 females), and their age varied between 34 and 67 years (mean± SD= 44.3 ±13.9). Group III included 60 patients with an established diagnosis of HCC in addition to HCV (42 males/ 18 females) whose age varied between 48 and 60 years (mean ± SD= 46.8 ± 15.9). Exclusion criteria have been established for the elimination of HBV comorbidity, schistosomiasis, autoimmune liver disease, alcohol intake or antiviral treatment from research. The informed consent of the patients who took part in the study was obtained in writing. In addition, the procedures used have been accepted by the ethics committee in the authors' institution according to the Helsinki Declaration.

Genomic DNA extraction

A QIAamp DNA Mini Kit (Qiagen; Cat No: 51104) was used to obtain genomic DNA. Five millilitres of peripheral venous whole blood were collected by means of a standard protocol using proteinase K in a sterilised vacuum tube containing EDTA for genomic DNA extraction. The lysis buffer was used to lyse the red blood

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cells. In the presence of guanidine HCL, 10 % sodium dodecyl sulphate (SDS) and 10 µl proteinase K were then added to treat the remaining white cells for a brief incubation period (10 minutes at 56ºC) in order to inactivate all nucleases. Cellular nucleic acids were then bound to special glass fibres prepacked in a high pure purification filter tube, and a sequence of "wash and spin" steps were used to remove PCR impurities using 500 µl AW1 buffer and 500 µl AW2 buffer. Lastly, to release nucleic acid from the glass fibre, an elution buffer (200 µl Buffer AE) was added and incubated at 15-25ºC for 1 minute.
XRCC3 CT gene polymorphism (rs861539) was detected using the TaqMan SNP genotyping assay. The primer pairs used for the XRCC3 Thr241Met (rs861539) locus amplification were as follows: sense primer: 5'-GGCCAGGCATCTGCAGTC-3', antisense primer: 5'-CAGCACAGGGCTCTGGAA-3'. Each PCR had 2.5 µl of diluted DNA (5 ng/µl), 12.5 µl of 2× TaqMan Universal PCR Master Mix, 1.25 µl of 20× TaqMan SNP Genotyping Assay Mix and 8.75 µl of distilled water. PCR amplification was performed with the ABI 7500 in a thermal cycler, with an initial step of 10 minutes at 95°C, followed by 40 cycles at annealing temperature of 95 ° C for 25 seconds, and 1 minute. Finally, for study using the ABI Prism Genetic Analyzer a threshold is set at 0.1. These procedures were used for quantification of the amplified PCR product (DNA fragments) and to determine the fragment size by comparing it to fragments contained in a standard size.

Statistical analysis

Microsoft Excel 2010 and the Social Science Statistical Program (SPSS version 22.0) for Windows (SPSS IBM., Chicago, IL) analysed the data. Continuous variables normally distributed were represented as mean ± SD with confidence interval of 95 %, the p-value of less than 0.05 was deemed statistically significant in addition to the use of alleles and genotype frequencies and percentage for categorical and non-parametric variables. Student's t-test was carried out to compare the means of variables normally distributed among groups. ANOVA followed by Tukey-Kramer as a multi-group post-hoc test, $\chi 2$ test or the exact Fisher test were used to determine distributing categorical variables among groups.

RESULTS

Patient characteristics
The demographic, clinical and serological characteristics of the 55 patients with HCV, 60 patients with HCC and the control group (n=50) are represented in Table 1.

Table 1: Demographic and Clinical Data of the study groups

Demographic and clinical data	Group I Control N=50	Group II HCV N=55	Group III HCC N=60
Sex N (%) M F	 36(72.0%) 14(28.0%)	 27(49.1%) 28(50.1%)	 42(70.0%) 18(30.0%)
Age (year)	46.7±13.3	44.3 ±13.9	46.8±15.9
TLC ($x10^3$/µl)	6.9 (5.4- 8.2)	7.7 (5.2- 10.1)	6.2 (4.9- 8.7)
HB (mg/dl)	12.9±1.1	9.6±1.7	10.4±1.8
Platelet ($x10^6$/µl)	305.8±100.7	118.6±31.5	145.8±64.4
PT (sec)	12.2±1.3	16.1±5.1	14.9±4.5
PC (%)	88.5±12.1	63.6±25.1	73.5±24.8
PTT (sec)	31.3±6.5	42.5±5.5	39.9±5.9
ALT (U/L)	28.5 (18.5- 35.1)	39.7 (24.1- 56.8)	49.1 (32.9- 72.8)
AST (U/L)	31.7 (24.0- 41.85)	54.9 (38- 79.1)	62.0 (41.5- 87.1)
Albumin (g/l)	4.1±0.8	2.7±4.1	2.8±1.0
T.Bilirubin (mg/l)	0..65(0.35- 1.1)	2.5 (1.4- 4.9)	1.7 (0.8- 2.9)
D.Bilirubin (mg/l)	0.15 (0.1- 0.35)	0.9 (0.6- 2.8)	0.8 (0.2- 1.8)

Sex is represented as number and percent. HB, PLT, PT, PC, PTT and albumin are represented as the mean ± *SD*. TLC, ALT, AST, T. Bilirubin and D. Bilirubin are represented as the *median* [interquartile range] (25%-75%).

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The studied patient groups were unmatched in sex ratio. The data was analysed separately based on sex. Additionally, AOR was performed to assess HCC risk based on *XRCC3* genotype stratified by sex (Table 2). Sex was not found to be a risk factor developing HCC based on *XRCC3* genotype.

Table 2: Relative risk for developing HCC based on *XRCC3* genotype stratified by sex in the studied groups:

Factors	*XRCC3 Polymorphism*		
	CC	***CT***	***TT***
HCV Cases Compared to Control Group			
Male			
No. of HCV/control	5/13	15/19	7/4
AOR (95%CI)	1 (reference)	0.923 (0.198 - 4.312)	0.333 (0.046 - 2.431)
P value	-	0.9	0.2
Female			
No. of HCV/control	6/4	13/8	9/2
AOR (95%CI)	1 (reference)	1.2 (0.335 - 4.524)	1.0 (0.248 – 3.789)
P value	-	0.7	0.9
HCC Cases Compared to HCV Group			
Male			
No. of HCC/HCV	8/5	15/15	19/7
AOR (95%CI)	1 (reference)	0.462 (0.096 - 2.224)	1.067 (0.233 - 4.885)
P value	-	0.3	0.9
Female			
No. of HCC/HCV	5/6	5/13	8/9
AOR (95%CI)	1 (reference)	0.625 (0.166 - 2.356)	0.873 (0.124 – 1.897)
P value	-	0.4	0.9

AOR: Adjusted odds ratio; *CI*: Confidence interval; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; No: Number; $^{*}P$ value ≤ 0.05 significant; $^{**}P$ value ≤ 0.01 highly significant.

The *XRCC3 CT* gene polymorphism (rs861539) was evaluated using RT- PCR. The results showed a highly significant difference in allele frequencies between both HCV and HCC patients when compared to control group (p=0.001), although the allele frequencies between HCC and HCV groups did not differ significantly (p=0.08).

The *CC* genotype frequency was higher among the control group (34.0%) compared with HCV subjects (20.0%) and HCC patients (21.7%). Similarly, the *CT* genotype frequency was higher among controls (54.0%), HCV patients (50.9%) and HCC patients (33.3%). The *TT* genotype frequency was higher among HCC patients (45%) and HCV patients (29.1%) compared to controls (12%).

Furthermore, the *C* allele was remarkably frequent among healthy individuals (0.61) compared to HCV patients (0.455) and HCC patients (0.383). On the other hand, the *T* allele frequency was higher among HCC patients (0.617) than HCV patients (0.545) and healthy individuals (0.39). Data are presented in Table 3.

Table 3: Genotype and allele frequencies of *XRCC3* among the studied groups

***XRCC3* Polymorphism**		**Groups**			**Total**	***P* value**		
		Group I Control N=50	**Group II HCV N=55**	**Group III HCC N=60**		**Control & HCV**	**Control & HCC**	**HCV & HCC**
***XRCC3* Polymorphism**	**Homo (wild) *CC***	17 (34.0%)	11 (20.0%)	13 (21.7%)	41	0.01*	0.02*	0.4
	Hetero *CT*	27 (54.0%)	28 (50.9%)	20 (33.3%)	75	0.4	0.001**	0.01*
	Homo *TT*	6 (12.0%)	16 (29.1%)	27 (45.0%)	49	0.01*	0.001**	0.02*
	CT* + *TT	33 (66.0%)	44 (80.0%)	47 (78.3%)	124	0.01*	0.02*	0.5
Total	**Allele *C***	61 (0.610)	50 (0.455)	46 (0.383)	157	0.001**	0.001**	0.08
	Allele *T*	39 (0.390)	60 (0.545)	74 (0.617)	173			

All parameters are represented as number and Percent; the data was analyzed by *x2* Test.

*: *P* value ≤0.05 (significant), **: *P* value ≤0.001 (highly significant).

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DISCUSSION

HCC is a major healthcare problem. It represents the fourth cancer-induced cause of death **[1]**. Its genetic origin has been a pivot of many studies over the last few years. The clinical course of hepatitis C could be affected by certain genotypic variants as elucidated by several studies, and an individual's predisposition to develop liver cancer plays key roles for HCC susceptibility in human phenotypic variability **[10]**.

Hepatitis virus is correlated with DNA damage, DNA damage response relies mainly on DNA damage repair (DDR) encoded enzyme pathways which has fundamental role in maintaining the stability of genomic DNA. One of critical DDR gene family member is the *XRCC3* gene **[11]**. Its protein product that plays an efficient role in double-strand DNA break/repair via a homologous recombination mechanism to maintain chromosome stability and repair DNA damage.**[8]**. Therefor XRCC3 critical for chromosomal integrity, depletion and reduction in its activity results in significant decreases in homologous recombination frequency and triggers carcinogenesis **[12]**. Moreover, *XRCC3* polymorphism may reduce its DRC **[13, 8]**. DDR genes has pivotal role in defencing against ionizing radiation damage, chelating agents and endogenous metabolic factors through nucleotide excision repair **[14]**.

The pathogenesis of HCC is multifactorial process one of the implicated factors is SNP in the XRCC3 gene (rs861539). This SNP leads to Thr241Met amino acid substitution. Accordingly, this substitution alters *XRCC3* function and/or interaction with other proteins involved in DDR **[12, 15]**. Despite that, relevance between *XRCC3* Thr241Met polymorphism and HCC susceptibility remains inconclusive **[16]**. The causes for such discrepancies emerged as most previous investigations focused on a small sample size single case-control studies.

The current study assessed the relationship between *XRCC3* T241M polymorphism and HCC risk in HCV-infected Egyptian patients. The retrieved data showed that the *CC* wild genotype frequency was higher among the control group (34.0%) than the HCV patients (20.0%) and HCC patients (21.7%). Similarly, the *CT* (Thr/Met) heterozygous genotype frequency was higher among controls (54.0%), HCV patients (50.9%) and HCC patients (33.3%), while the *TT* genotype frequency was higher among HCC patients (45%) than HCV patients (29.1%) and control (12%). Collectively, the *T allele* frequency was higher among HCC patients (0.617) compared to HCV patients (0.545) and healthy individuals (0.39), which indicates that *XRCC3* Thr241Met polymorphism exerted elevated risk effect on the HCC pathogenesis between HCV positive people. This agrees with **Zeng *et al.* [13]** and **Golnaz *et al.* [17]**, who reported that *XRCC3* Thr241Met was associated with liver cancer in the Pakistani population, in addition to **Duan *et al.* [16]**, who reported a high risk of HCC with *XRCC3* 241 *TT* genotypes.

Similarly, these results agree with that of **De Mattia *et al.* [18]** and **Yao *et al.* [19],** who reported that a higher frequency of genotypes with *XRCC3* codon 241*T allele* (namely, Thr/Met and Met/Met) was observed in HCC cases. Regression analysis showed that the *T allele* confers an approximately 2- to 10-fold risk of HCC and the number of T alleles (adjusted OR 2.48 and 10.06) for one and two alleles modulates this increase in risk.

In more recent study done by **Zhao et al [1],** who performed comprehensive meta-analysis to assess the correlation between XRCC1, XRCC3 and ERCC2 gene polymorphism and prognosis of HCC, they concluded that ERCC2 rs13181 A>C polymorphism and XRCC3 rs861539 C>T polymorphisms are possible HCC prognostic markers.

The study by **Zeng *et al.*** found that the *XRCC3*-241 wild-type *CC* genotype was a risk factor for low DRC. These results conflict with the current study. The key explanation for this discrepancy could be the disparity in research samples and ethnic specific phenotypic effects **[13]**.

Another conflicting results obtained by **Avadani et al [21]** who studied the relationship between the *XRCC3* gene SNPs and HCC clinicopathological features, and its role in influencing the tumor behavior, they found no significant association between rs861539 polymorphism and HCC risk. But their findings indicate that XRCC3 gene SNPs may affect the aggressiveness of tumors and better survival for the homozygote genotype (TT)

CONCLUSIONS

Our data suggest that *XRCC3 CT* and *TT* polymorphism could reduce the DRC and increase the risk of HCC. *XRCC3 CT* gene polymorphism (rs861539) might play a role in predicting the susceptibility of HCC in a cohort of a high-risk (HCV-infected) population compared to healthy Egyptian population. However, the exact molecular mechanism of this variant is currently unknown. One limitation of this study is that we could not exclude a linkage with other functional markers. These preliminary findings may have important clinical implications in managing HCC prevention and patient care, although confirmation is needed.

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This study highlights the need for more high-quality case-control studies to evaluate the possible role of gene-gene and gene-environment interactions in hepatocarcinogenesis in promoting of HCC diagnosis, therapy and prevention.

Declarations:

Ethics approval and consent to participate
A written informed consent from patients who participated in this study was obtained. In addition, TBRI ethics committee approved the procedures used in accordance with the Helsinki Declaration (approval number: 00010609).

Consent for publication
This study was approved by the Hematology laboratory board and
Clinical laboratory research division, TBRI, Giza, Egypt.

Availability of data and material
Available.

Competing interests:
The authors declare no competitive interests.

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Authors contributions:
BM performed the molecular genetic studies, took part in the alignment of sequences and drafted the manuscript. MZ performed the immunoassays and took part in the alignment of sequences. OS and MW took part in the study design and conducted the statistical analysis, coordination and helped to draft the manuscript. MA and AH
provided clinical management to patients. The final Manuscript was read and accepted by all authors.

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List Of abbreviations:
Hepatocellular carcinoma (HCC), Hepatitis C virus infection (HCV), The Egyptian Demographic and Health Surveys (EDHS), Direct-acting antiviral (DAA) treatments, DNA repair capacity (DRC), X-ray repair cross-complementing group 3 (*XRCC3*) gene, Schistosoma antibodies (Sch Ab) , Chronic liver diseases (CLD), Non-alcoholic steatohepatitis (NASH), Sodium dodecyl sulphate (SDS), Ethylenediaminetetraacetic acid (EDTA), Single nucleotide polymorphisms (SNPs), DNA damage response (DDR) genes, Direct-acting antiviral drugs (DAAT)

REFERENCES

1. **ZhaoY, Zhao E, Zhang J, Chen Y , Junli Ma J , and Li H (2019):** A Comprehensive Evaluation of the Association between Polymorphisms in XRCC1, ERCC2, and XRCC3 and Prognosis in Hepatocellular Carcinoma: A Meta-Analysis Journal of Oncology, Volume 2019, Article ID 2408946, 8 pages
2. **Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu M, *et al.*, (2017):** Global burden of disease liver cancer collaboration, the burden of primary liver cancer and underlying aetiologies from 1990 to 2015 at the global, regional, and national level results from the global burden of disease study 2015. *JAMA Oncol;* 3:1683-1691.
3. **Abd-Elsalam S, Elwan N, Soliman H, Ziada D, Elkhalawany W *et al.*, (2018):** Epidemiology of liver cancer in Nile delta over a decade: a single-center study. *South Asian J Cancer;* 7:24-26.
4. **Elghazaly H, Gaballah A, Bahie Eldin N (2018):** P-019 Clinic-pathological pattern of hepatocellular carcinoma (HCC) in Egypt. *Annals of Oncology;* 29 suppl_5. mdy151.018, https://doi.org/10.1093/annonc/mdy151.018.
5. **Nahon P and Zucman-Rossi J (2012):** Single nucleotide polymorphisms and risk of hepatocellular carcinoma in cirrhosis. *J Hepatol;* 57: 663-674.

ISSN- 2394-5125 VOL 7, ISSUE 11, 2020

6. **Kouyoumjiam S, Chemaitelly H and Abu-Raddad L (2018):** Characterizing hepatitis C virus epidemiology in Egypt: systematic reviews, meta-analyses, and meta-regressions. *Scientific Reports;* 8:1661-1670.
7. **Shi Y, Wang B, Xu B, Jiang D, Zhao D et al., (2016):** The association of six non-synonymous variants in three DNA repair genes with hepatocellular carcinoma risk: a meta-analysis. *J Cell Mol Med;* 20 (11): 2056-2063.
8. **Roderburg C, Tacke F and Trautwein C (2016):** Antiviral therapy in patients with viral hepatitis and hepatocellular carcinoma: indications and prognosis. *Visc Med;* 32: 121-126.
9. **Ji R, Qian A and Hu Y (2015):** DNA repair gene *XRCC3* T241M polymorphism and susceptibility to hepatocellular carcinoma in a Chinese population: a meta-analysis. *Genetics and Molecular Research;* 14 (4): 15988-15995.
10. **McGlynn K, Petrick J and London W (2015):** Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability. *Clin Liver Dis;* 19:223-238.
11. **Matsuura K and Tanaka Y (2016):** Host genetic variants influencing the clinical course of hepatitis C virus infection. *J Med Virol;* 88: 185-195.
12. **Cheng X, Li K and Li W (2012):** Predictive value of *XRCC1* and *XRCC3* gene polymorphisms for risk of ovarian cancer death after chemotherapy. *Asian Pacific J Cancer Prevention;* 13 (6): 2541-2545.
13. **Matullo G, Palli D, Peluso M, Guarrera S, Carturan S *et al.*, (2001):** *XRCC1, XRCC3, XPD* gene polymorphisms, smoking and (32)P-DNA adducts in a sample of healthy subjects. *Carcinogenesis;* 22 (8):1437-1445.
14. **Zeng X, Liu S, Yu H, Ji L, Li L, Huang J, *et al.*, (2012):** DNA repair capacity, DNA-strand break repair gene polymorphisms, and the incidence of hepatocellular carcinoma in Southwestern Guangxi of China. *DNA and Cell Biology;* 31 (8): 122-131.
15. **Avanade E, Giusca S, Negura L and Caruntu I (2018):** Single nucleotide polymorphisms of *XRCC3* gene in hepatocellular carcinoma – relationship with clinicopathological features. *Polish J Pathol;* 69(1): 73-81 https://doi.org/10.5114/pjp.2018.75340.
16. **Zhan P, Wang Q, Qian Q and Yu K (2013):** *XRCC3* Thr241Met gene polymorphisms and lung cancer risk: a meta-analysis. *J Exp & Clin Cancer Res;* 32 (1):1201-1213.
17. **Duan C, Zhang W, Lu J, Huawen H, Liu M and Zhu W (2013):** DNA repair gene *XRCC3* Thr241Met polymorphism and hepatocellular carcinoma risk. *Tumor Biol;* 34:2827-2834.
18. **Gulnaz A, Sayyed A, Amin F, Khan A, Aslam M, *et al.*, (2013):** Association of *XRCC1, XRCC3* and *XPD* genetic polymorphism with an increased risk of hepatocellular carcinoma because of the hepatitis B and C virus. *Eur J Gastroenterol Hepatol;* 25(2):166-179.
19. **De Mattia E, Cecchin E, Polesel J, Bignucolo A and Roncato R (2017):** Genetic biomarkers for hepatocellular cancer risk in a Caucasian population. *World J Gastroenterol*; 23(36): 6549-6562.
20. **Yao J, Huang X and Long X (2014):** Interaction of DNA repair gene polymorphisms and aflatoxin B1 in the risk of hepatocellular carcinoma. *Int J Clin Exp Pathol;* 7(9):6231-6244.
21. **E.-R. Avadanei, S.-E. Giusca, L. Negura, and I.-D. Caruntu (2018)** "Single nucleotide polymorphisms of XRCC3 gene in hepatocellular carcinoma - relationship with clinicopathological features Polish Journal of Pathology, vol. 69, no. 1, pp. 73–8