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EVALUATION OF 40-bp DELETION / INSERTION POLYMORPHISM OF mdm2 AND 16-bp DELETION / INSERTION POLYMORPHISM OF p53 GENE IN PATIENTS WITH LYMPHOMA IN A PERSIAN POPULATION

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Considering the importance of lymphoma and its prevalence in communities as well as its relationship with genetic factors (P53 & MDM2) as well as contradictory results about the possible role of deletion / insertion of polymorphisms in different types of cancer. The aim of this study was to investigate the deletion / insertion of 40 bp of mdm2

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polymorphism and 16-bp of p53 polymorphism in patients with lymphoma. In this case-control study, 152 non-Hodgkin's lymphoma patients and 155 healthy individuals were selected by convenience sampling method. MDM2 and P53 polymorphisms were examined by PCR. SPSS V22 software was used to interpret the results. The results of the study showed that rs3730485 species was associated with the risk of lymphoma. The Ins / Del and Del / Del genotypes reduce the risk of lymphoma compared to the Ins / Ins genotype (OR = 0.41,95% CI = 0.25-0.65 P <0.001 and OR = 0.33.95% CI = 0.12). 0.96, P = 0.035 In relation to Ins / Del + Del / Del and Ins / Del, the results also showed that they reduce the risk of lymphoma (OR = 0.40,95% CI = 0.25-0.63, P <0.001). Results indicated that Ins / Del, Del / Del, Ins / Del + Del / Del and D alleles genotypes had a statistically significant relationship with lymphoma risk. Due to the fact that the OR genotypes of this site are less than one step away, it is a protective factor for lymphoma, so that these alleles and the mentioned genotype reduce the risk of lymphoma.

Keywords: Humans; Tumor Suppressor Protein p53; Polymorphism, Genetic; Neoplasms; Genotype; Lymphoma

INTRODUCTION

In general, lymphoma covers a wide range of cancers (with different causes, genetic characteristics, phenotypes, different involvement pathways, and different treatments and outcomes) (NIINO *et al.*, 2021). The prevalence of lymphoma varied around the world, with 73.27 non-Hodgkin's lymphoma in the United States and 2.4 per 100,000 in China in 2008. Hodgkin's lymphoma also had a prevalence of 88.4 per 100,000 in the UK and 0.8 per 100,000 in Japan in 2008. In general, the prevalence of Hodgkin's lymphoma is higher than Hodgkin's lymphoma (HERBER *et al*., 2020, HUH, 2012). According to the WHO classification, the classification of lymphoid neoplasms includes not only the same lymphoma and non-Hodgkin's lymphoma, but also the neoplasm of plasma cells and leukemia lymphoma (HARRIS *et al*., 2000). The p53 transcription factor is encoded by the p53 tumor inhibitor gene, which plays an important role in the modulation and regulation of cellular stress (PFLAUM *et al.*, 2014). It is a cell that can act as a barrier to cell growth and cause cell death during injury (AUBREY *et al.*, 2018). Due to the prominent role of p53 as a tumor suppressor, any mutation or deletion in the p53 gene range has been proven in up to 50% of human cancers (JALILIAN *et al*., 2021). In most human cancers, various mutations or misstatements of P53 pathway proteins, such as MDM2, are observed (MARTINS *et al*., 2021). The MDM2 gene is located on chromosome 12q14.3-15. MDM2 is an inhibitory factor in P53, which is encoded by the MDM2 gene and its direct function is to inhibit transcriptional activity of the P53 gene (KONOPLEVA *et al*., 2020). In human cancer cells, wild-type P53 is present in the cell but the function of this P53 is inhibited by MDM2 (LOU *et al.*, 2020). MDM2 is a modulator and regulator of P53 in normal cells of the body, but overexpression of this gene increases the unrestrained growth of cells (BARRIO *et al.*, 2021). MDM2 and P53 generally modulate each other through a cyclic regulatory chain. Once activated, p53 activates and transduces the MDM2 gene, and thus the MDM2 protein inhibits p53 activity. MDM2 binds to the transcription region of the p53 gene and inhibits its transcriptional activity (CHEN *et al*., 2021). Numerous studies in the past have shown an

association between MDM2 polymorphism and cancer risk in cells (GAO *et al.*, 2014, ZHUO *et al*., 2014, WANG *et al.,* 2014). Numerous studies have evaluated the association between changes in bases and polymorphisms in MDM2 and the risk of various cancers (DONG *et al*., 2012, HU *et al*., 2006, MA *et al*., 2006).

Considering the importance of lymphoma and its prevalence in communities as well as its relationship with genetic factors (P53 & MDM2) as well as contradictory results about the possible role of deletion and attachment of bases in different types of cancer and not examining this type of deletion / attachment in different types of lymphoma. We decided to investigate the role of these factors in lymphoma patients in lymphoma by examining the removal/ incorporation of 40 bp of mdm2 and the polymorphism of removal / incorporation of 16 bp in the p53 gene in patients with lymphoma and to identify communication factors.

MATERIALS AND METHODS

Study population

In this case-control study, 152lymphoma patients and 155 healthy individuals were evaluated. This study was performed in the teaching hospital of Zahedan University of Medical Sciences in southeastern Iran in 2020. Inclusion criteria were being over 18 years of age and having a known lymphoma and being treated. People who did not agree to participate left. The control group included healthy non-lymphoma patients who did not have systemic disease. Patients were selected by convenience sampling method. The most important outcomes of this study were: determining the frequency of polymorphism of removal / insertion of 40 bp of mdm2 in patients with lymphoma and healthy individuals and determining the frequency of polymorphism of removal / incorporation of 16 bp of p53 gene in patients with lymphoma and healthy individuals. To determine the sample size, a previous similar study was performed on patients with lymphoma (DONG, 2012). The number of people in the two groups was equal. Demographic characteristics collected included age and gender.

Data collection

First, the objectives of the study were explained separately to patients and healthy individuals, and after their consent, they entered the study. Participants' DNA samples were extracted by salting out method. Samples were kept at -20 ° C until analysis. Primer fragments of P53 16-bp I / D and MDM2 40-bp I / D used in this study are shown in Table 1. PCR primer was prepared in 20 microliters, which included: 1 µl of DNA genome, 10 µl of 2X prime (Genetbio from South Korea) and 1 µl of any other primer and 7 µl of ddH2O. PCR conditions for P53 16-bp I / D were as follows: at the beginning of the denaturation stage, the temperature was set at 95 ° C for 5 minutes, then at a temperature of 30 ° C for 30 seconds. In the annealing stage, the temperature was 59 degrees for 25 seconds. In the Extension stage, we set the temperature to 72 degrees for 30 seconds and then to the temperature of 72 degrees for 10 minutes. PCR conditions for MDM2 40-bp I / D were as follows: at the beginning of the denaturation stage, the temperature was set at 95 ° C for 5 minutes, then at 30 ° C for 30 minutes. In the annealing stage,

the temperature was 60 degrees for 30 seconds. In the Extension stage, we set the temperature to 72 degrees for 30 seconds and then to the temperature of 72 degrees for 10 minutes. PCR products were isolated by agarose electrophoresis gel and exposed by UV.

Table 1. The primers used for detection of P53 16-bp I/D and MDM2 40-bp I/D genes polymorphism

Polymorphism	Primer sequence	Fragment(bp)
TP53 16-bp I/D	F:CTGAAAACAACOTTCTGGTA	Ins-135
	R:AAGGGGGACTGTAGATGGGTG	Del-119
MDM2 40-BP I/D	F:GACCACTATGTITAAGGAAG	Ins-287
	R:TGACTCACCTACTTTCCCAC	Del-119

Ethical considerations

This study was approved in the ethics committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1399.398). Written and oral informed consent was obtained from all participants. The participants were assured about the confidentiality of their information.

Data Analysis

Extracted data was analyzed using SPSS software version 25 for analysis. Descriptive statistics including statistical tables and graphs, frequencies and percentages were used to describe the data, and t-test and $\chi 2$ test were used to compare the frequencies for data analysis. The genotype distribution of the Hardy-Weinberg equilibrium model HWE was examined separately for case and control cases. The significance level in this test was less than 0.05.

RESULTS

In this study, among 152 patients with non-Hodgkin's lymphoma (77 males and 75 females) and among 155 patients in the control group (74 males and 81 females). The mean age of the case and control groups was 16.57. 45.51 And 12.06 43 43.25, respectively.

The polymorphisms of MDM2 40-bp I / D and P53 16-bp I / D genotypes are shown in Table 2.

According to the results of this study, rs3730485 species is associated with the risk of lymphoma. Ins / Del and Del / Del genotypes reduce the risk of lymphoma compared to Ins / Ins genotype (OR = 0.41,95% CI = 0.25-0.65; OR = 0.33, 95% CI = 0.12, respectively) (P<0.0010). In Ins / Del + Del / Del and Ins / Del reduce the risk of lymphoma (OR = 0.40.95% CI = 0.25-0.63, P <0.001).The allele in rs17878362 polymorphism reduced the risk of non-Hodgkin's lymphoma (OR = 0.54.95% CI = 0.38-0.77, P <0.001), so P53 16-bp I / D (rs17878362) had no association between genotypes and alleles. Did not indicate a risk or safety against non-Hodgkin's lymphoma. (Table 2).

Table 2. Genotypic and allelic frequencies of the TP53 and MDM2 40-bp I/D Polymorphism in patients with non-Hodgkin lymphoma and controls

Polymorphism	Case n(%)	Control n (%)	OR(95%CI)	P
TP53 16-bp I/D (rs17878362)				
Codominant				
Del/Del	27(17.8)	28(18.1)	1.00	
Del/Ins	116(76.3)	121(78)	0.99(0.55-1.79)	0.984
Ins/Ins	9(5.9)	6(3.9)	1.56(0.49-4.96)	0.456
Dominant				
Del/Del	27(17.8)	28(18.1)	1.00	
Del/Ins/ins/ins	125(82.2)	127(81.9)	1.02(0.57-1.83)	0.945
Receccive				
Del/del+del/Ins	143(94.1)	149(96.1)	1.00	
Ins/Ins	9(5.9)	6(3.9)	1.56(0.54-4.50)	0.406
Allele				
Del	170(55.9)	177(57.1)	1.00	
Ins	134(44.1)	133(42.9)	1.05(0.76-1.44)	0.769
MDM2 40-BP I/D (rs3730485)				
Codaminiant				
Ins/Ins	85(55.9)	52(33.5)	1.00	
Ins/Del	61(40.1)	92(59.4)	0.41(0.25-0.65)	<0.001
Del/del	6(4)	11(7.1)	0.33(0.12-0.96)	0.035
Dominaant				
Ins/Ins	85(55.9)	52(33.5)	1.00	
Ins/del+Del/del	67(44.1)	103(66.5)	0.40(0.25-0.63)	<0.001
Reccecive				
Ins/Ins+Ins/Del	146(96)	144(92.9)	1	
Del/Del	6(4)	11(7.1)	0.53(0.19-1.49)	<0.001
Allele				
Ins	231(76)	196(63.2)	1	
Del	73(24)	114(36.8)	0.54(0.38-0.77)	<0.001

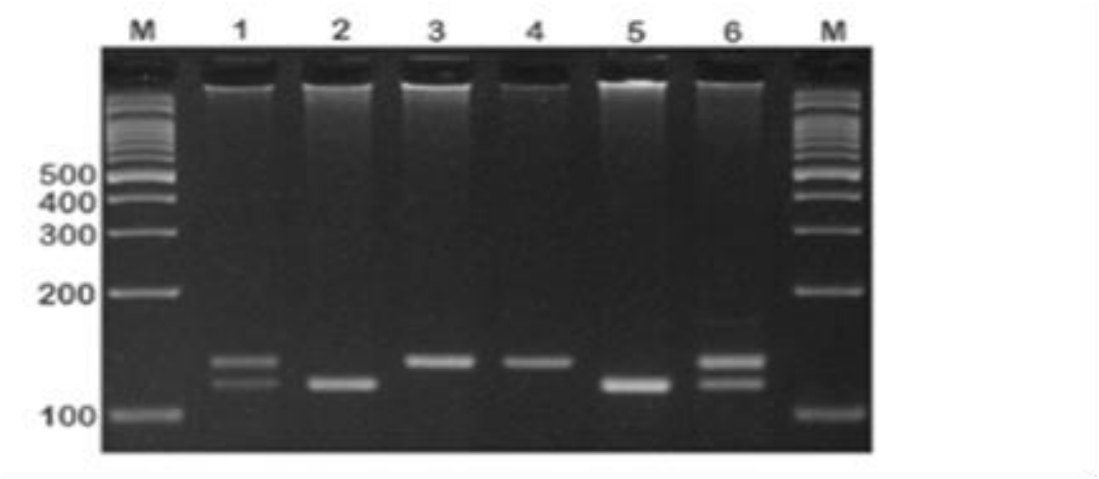

Figure 1. Photograp of electrophoresis pattern of TP53 16-bp I/D polymorhism M: DNA marker, lanes 1and 6: Ins/Del:lanes 2 and 5: Deel/del:lanes 3 and 4: Ins/Ins

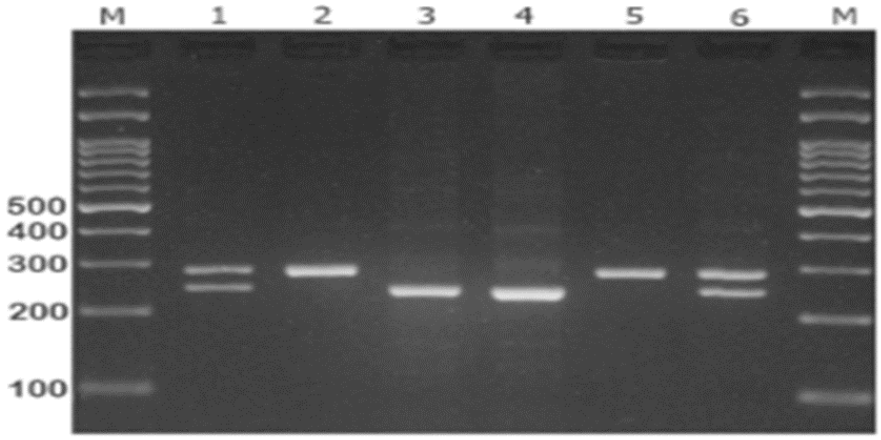

Figure 2. Photograp of electrophoresis pattern of MDM2 40-bp I/D polymorhism M: DNA marker, lanes 1and 6: Ins/Del:lanes 2 and 5: Ins/Ins:lanes 3 and 4: Del/del

DISCUSSION

Epidemiological studies are a very cost-effective way to assess the relationship between disease risk and gene changes. So far, most studies with emphasis on intronic promoter polymorphisms have been like rs17878362 but fewer studies on polymorphisms constitutive promoter of MDM2.

The aim of this study was to evaluate the deletion / insertion polymorphism of 40 bp of MDM2 and the polymorphism of deletion / insertion of 16 bp of p53 gene in patients with lymphoma and compare it with healthy individuals. In our study of deletion / incorporation of p53 and deletion of annexation of MDM2, statistical results showed that the genotypes Ins / Del, Del / Del, Ins / Del + Del / Del and D alleles had a statistically significant relationship with the risk of lymphoma. Due to the fact that OR genotypes of this site are a protective factor for lymphoma, so that these alleles and the mentioned genotype reduce the risk of lymphoma.

In our study, it was found that rs3730485 species can cause lymphoma and increase the risk of developing this disease. In a study by HASHEMI *et al*., There was no significant association between deletion polymorphism and the incorporation of 16 bp into prostate cancer. Also, the present study showed that the removal / incorporation polymorphism of 40 bp in the MDM2 promoter increases the risk of prostate cancer in an Iranian population, which is consistent with the results of our study (MA *et al*., 2006).

The results of AUBREY *et al*., (2014) study show the positive and important role of P53 in preventing people from developing MYD-C lymphoma. Also, different p53 mutations have different functional characteristics, so that different p53 mutations are probably associated with different risks. These results were consistent with our study and in our study it was also stated that rs3730485 species reduces the risk. Lymphoma and rs17878362 were not associated with lymphoma. In the study of HUA *et al*. (2017) it was observed that there is no association between 18MDM2 / del15 polymorphism and the risk of cancer in humans, which was not consistent with the results of our study, which can be attributed to the sample size. The higher the sample size done the more the results will change.

In a study by DONG *et al*., (2012) it was concluded that MDM2 polymorphism may be a modifier of cellular genetics; For people with liver cell cancer, which was completely consistent with the results of our study. The most important limitation of the present study was the small sample size. Future studies should be performed on a larger sample size.

CONCLUSION

In our study results showed that Ins / Del, Del / Del, Ins / Del + Del / Del and D alleles genotypes had a statistically significant relationship with lymphoma risk. Due to the fact that the OR genotypes of this site are less than one step away, it is a protective factor for lymphoma, so that these alleles and the mentioned genotype reduce the risk of lymphoma. In our study, it was found that rs3730485 species can cause lymphoma and increase the risk of developing this disease. However, similar studies in other populations with diverse demographic characteristics are still recommended.

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EVALUACIJA POLIMORFIZMA 40-bp DELECIJE/ INSERCIJE mdm2 I POLIMORFIZMA 16-bp DELECIJE/ INSERCIJE p53 GENA KOD PACIJENATA SA LIMFOMOM U PERSIJSKOJ POPULACIJI

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Izvod

Uzimajući u obzir značaj limfoma i njegovu prevalenciju u zajednicama, kao i njegov odnos sa genetskim faktorima (P53 & MDM2), kao i kontradiktorne rezultate o mogućoj ulozi brisanja/ umetanja polimorfizama u različitim vrstama karcinoma. Cilj ove studije je bio da se ispita delecija / insercija 40 bp polimorfizma mdm2 i 16-bp polimorfizma p53 kod pacijenata sa limfomom. U ovoj studiji kontrole slučaja, 152 pacijenta sa non- Hodgkinovim limfomom i 155 zdravih pojedinaca odabrano je pogodnom metodom uzorkovanja. Polimorfizmi MDM2 i P53 ispitani su PCR -om. Za tumačenje rezultata korišćen je softver SPSS V22. Rezultati studije su pokazali da je vrsta rs3730485 povezana sa rizikom od limfoma. Genotipovi Ins / Del i Del / Del smanjuju rizik od limfoma u poređenju sa genotipom Ins / Ins (OR = 0,41,95% CI = 0,25-0,65 P <0,001 i OR = 0,33,95% CI = 0,12). 0,96, P = 0,035 U odnosu na Ins / Del + Del / Del i Ins / Del, rezultati su takođe pokazali da smanjuju rizik od limfoma (OR = 0,40,95% CI = 0,25-0,63, P <0,001). Rezultati su pokazali da genotipovi alela Ins / Del, Del / Del, Ins / Del + Del / Del i D imaju statistički značajnu vezu sa rizikom od limfoma. Zbog činjenice da su ORgenotipovi ove lokacije udaljeni manje od jednog koraka, on je zaštitni faktor za limfom, tako da ti aleli i pomenuti genotip smanjuju rizik od limfoma.

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