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RESEARCH ARTICLE

GENOTYPING OF P53 EXON 4 CODON 72 IN SUDANESE PATIENTS WITH LYMPHOID LEUKAEMIAS

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ABSTRACT

The aim of this was to investigate the association between P53 exon 4 codon 72 genotypes and acute and chronic lymphoid leukaemias. A total of 77 subjects were enrolled in this study, 32 with acute lymphoblastic leukaemia, 15 with chronic lymphocytic leukemia and 30 healthy individuals as a control group. Genomic DNA was extracted from patients' blood samples by salting out method, and analyzed for determination of P53 exon 4 codon 72 genotypes using-allele specific polymerase chain reaction (AS-PCR). The results showed that, the genotype Arg/Pro was the most frequent (75%) in patients with ALL, followed by the genotype Arg/Arg (19%), and Pro/Pro (6%). Also in patients with CLL the genotype Arg/Pro was the most prevalent (67%), followed by Arg/Arg (27%), and Pro/Pro (6%). In healthy control the Arg/Arg genotype was the most frequent genotype (80%), followed by Arg/Pro (17%), and Pro/Pro (3%). There was a significant association between the Arg/Pro genotype and both ALL (OR: 4.5, CI: 1.97-10.27, *P .value*: 0.000) and CLL (OR: 4.0, CI: 1.7-9.6, *P.value*:0.001). In conclusion, Arg/Pro genotype might confer increased risk for development of ALL and CLL.

INTRODUCTION

Leukaemias are very heterogeneous diseases with respect to clinical features and acquired genetic alterations. The etiology of Leukaemia as appears to be multi-factorial, including the inherited mutations in DNA, and exposure to ionizing radiation, or to chemicals like benzene or cytotoxic therapy. Exposure to these carcinogens may cause DNA damage at the level of hematopoietic progenitors and develop leukemia; however, the majority of cases likely involve genetic variations with a high-risk phenotype (Wengl *et al*., 2012). The P53 tumor suppressor gene, located on short arm chromosome 17p13, is one of the most commonly mutated genes in all types of human cancers (He *et al*., 2011; Chen *et al*., 2008). The P53 protein is a transcription factor that regulates the expression of a wide variety of genes involved in cell cycle arrest and apoptosis in response to genotoxic or cellular stress (Donehower and Bardley 1993; Ko and Prives, 1996). Growth arrest or cell death prevents damaged DNA from being replicated suggesting important role played by P53 in maintaining the integrity of the genome (Lane and Benchimol, 1990). The loss of functional P53 during tumorogenesis likely to represent an essential step in the switch to an angiogenic phenotype that was displayed by aggressive tumors (Teodoro *et al*., 2007). Several polymorphisms have been identified within P53 gene, both in non-coding and coding regions (Olivier *et al*., 2002). A common polymorphism occurs at codon 72 of exon 4, with two alleles encoding either arginine (CGC) or proline (CCC) (Zhang *et al*., 2003). This single nucleotide polymorphism appear to be different both biochemically and biologically (Dumont *et al*., 2003; Marin *et al.,* 2000). The arginine (Arg 72) allele increases the ability of P53 to locate to mitochondria and induce cellular death, whereas proline allele (Pro 72) exhibits a lower apoptotic potential and an increased cellular arrest in G1 of the cell cycle **(**Bergamaschi *et al*., 2004). This P53 polymorphism is reportedly associated with cancer susceptibility. The distribution of the three genotypes (Arg/Arg, Arg/Pro and Pro/Pro) depends largely on the ethnic composition of the studied population (Omori *et al*., 2004). Recent studies concerning with the relation between p53 gene polymorphism with cancers of the stomach, lung, breast, ovary, oral, cervix and leukemia showed conflict results (Yi and Lee, 2006; Wang *et al*., 1999; Boroujeni1 *et al*., 2012; Agorastos *et al*., 2004; Li1 *et al*., 2002; Tandle1 *et al*., 2001; Dunna *et al*., 2012; Jiang *et al*., 2010). In this context, we conducted a case-control study to examine the association between P53 exon 4 codon 72 Arg/Pro genotypes and the risk of acute and chronic lymphoid leukaemias among Sudanese patients.

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MATERIALS AND METHODS

Study population

A total of 47 Sudanese leukaemic patients admitted to Radiation and Isotopes Center of Khartoum (RISK) during the

period from July to September 2013 were enrolled in this study, 32 patients (31 male and 1 female) with Acute Lymphoblastic Leukemia (ALL) and 15 (13 male and 2 female) with Chronic Lymphocytic Leukemia (CLL). In addition, 30 healthy individuals were used as a control group.

Sample collection and DNA extraction

Blood samples were collected from all patients and control group in Ethylene Diamine Tetra Acetic Acid (EDTA) and genomic DNA was extracted by salting out method.

Polymerase Chain Reaction (PCR)

Analysis of the P53 exon 4 codon 72 genotype was performed by Allele-Specific PCR (AS-PCR). The primer sequences used were as follow:

Pro specific primers:

Sense primer: 5' GCC AGA GGC TGCTCC CCC 3'
Antisense primer: 5' CGT GCAAGT CAC AGA CTT 3'

Arg specific primers:

Sense primer: 5' TCC CCC TTG CCG TCC CAA 3'
Antisense primer: 5' CTG GTG CAG GGG CCA CGC 3'.

PCR reaction mixture of 20 µl was prepared for each sample. It consists of 3 µl of genomic DNA, 0.5 µl of each primer, 4 µl of "5X FIREPoL" ready to load master mix (SOLIS BIODYNE, ESTONIA) and 12 µl distilled water. Thermocycling conditions for Arg allele include initial denaturation at 94^0 C for 3 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60.0°C for 30 seconds and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes. Thermocycling conditions for Pro allele were similar to Arg allele except that, annealing temperature was 54°C. After amplification, PCR products and 50 bp DNA ladder (SOLIS BIODYNE, ESTONIA) were run on 3% agarose gel containing ethidium bromide and identified under UV transilluminator using gel documentation system (SYNGENE, JAPAN)

Statistical analysis

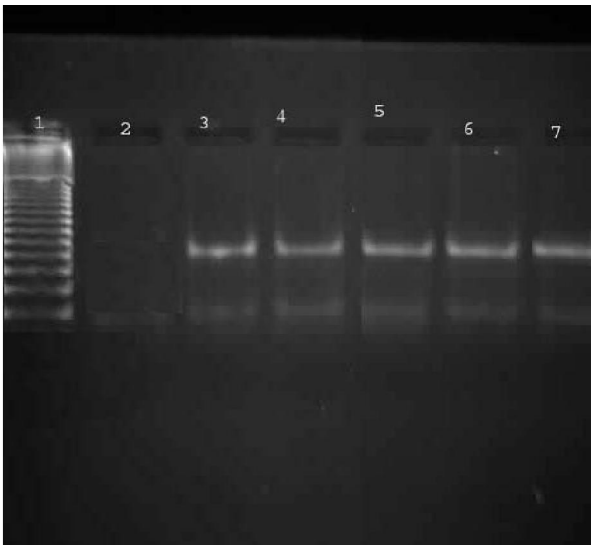
Data of this study was collected by structured interview questionnaire and from patients' medical files, and analyzed using Statistical Package for Social Sciences (SPSS). Frequencies of different genotypes were calculated, and correlation of genotypes with study groups was calculated by Chi-square test. The Hardy–Weinberg equilibrium was tested by a goodness-of-fit X^2 test to compare the observed genotypic frequencies in normal individuals to the expected genotypic frequencies calculated from the observed allelic frequencies.

Ethical considerations

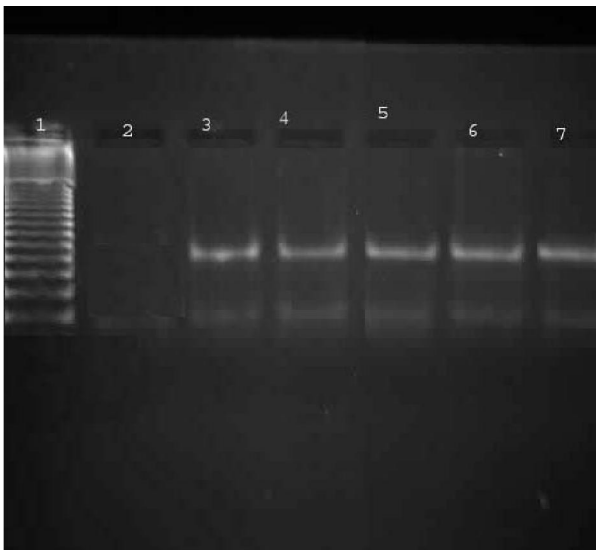
This study was approved by RICK and faculty of medical laboratory sciences, Al Neelain University, and informed consent was obtained from each patient before sample collection.

RESULTS

A total of 47 Sudanese patients were enrolled in this study, 32(68%) with ALL and 15(32%) with CLL. 31(96.8%) of patients with ALL were males and 1(3.2%) was female. Of those with CLL 13(86.7%) were males and 2(13.3%) were females. Further 30 healthy individual were included as a control group. Genotyping of P53 exon 4 codon72 was performed by AS-PCR. As shown in figure (1) the size of the amplified fragment of Pro allele was 177 bp; whereas Arg allele demonstrated a 141 bp fragment. The genotype Arg/Pro was the most frequent (75%) in patients with ALL, followed by the genotype Arg/Arg (19%) and Pro/Pro (6%). Also in patients with CLL the genotype Arg/Pro was the most prevalent (67%), followed by Arg/Arg (27%) and Pro/Pro (6%). In healthy controls the Arg/Arg genotype was the most frequent genotype (80%), followed by Arg/Pro (17%) and Pro/Pro (3%). There was a significant association between the



(A)



(B)

Figure 1. PCR amplification of the TP53 codon 72 sequences. PCR products were electrophoresed through a 3% agarose gel from genomic DNA from (A) Arg allelefrgment (B) Pro allele fragment

Arg/Pro genotype and both ALL (OR: 4.5, CI: 1.97-10.27, *P.value*: 0.000) and CLL (OR: 4.0, CI: 1.7-9.6, *P.value*:0.001). Arg allele frequencies were 0.56 in patients with ALL, 0.60 in patients with CLL, and 0.88 in control group; while Pro allele frequencies were 0.44 in patients with ALL, 0.40 in patients with CLL, and 0.12 in control group. However, a significant deviation from the Hardy–Weinberg equilibrium was observed in patients with ALL (X^2= 8.79, df=2 and P=0.012) but not in patients with CLL (X^2= 2.3, df=2 and P= 0.32) and control group (X^2= 1.09, df=2 and P= 0.58).

DISCUSSION

A common polymorphism in *TP53* gene, unique to humans is located within the proline rich region of *TP53* gene at codon 72 in exon 4 and encodes protein either with proline or arginine (Zhang *et al*., 2003). A recent study demonstrated the influence of *TP53 codon72* polymorphism on DNA repair capacity indicating that *TP53* 72 Pro variant activates several *TP53* dependent target genes involved in DNA repair more efficiently than the 72 Arg variant expressing cells (Siddique *et al*., 2005). It has been suggested that the *TP53 codon 72* polymorphism may influence expression of the *TP53* gene since the substitution occurs within the *TP53* transactivation domain (Gottlieb and Moshe, 1998; Wang *et al*., 1999). In this study, we examined the prevalence of P53 codon 72 genotypes in Sudanese ALL and CLL patients with comparison to control group. Our results showed that Arg/Pro genotype was the most common among patients with ALL and CLL followed by Arg/Arg and Pro/Pro consequently. In control group Arg/Arg was the most common genotype, followed by Arg/Pro and Pro/Pro consequently. There was a significant association between the Arg/Pro genotype and both ALL (OR: 4.5, CI: 1.97-10.27, *P .value*: 0.000) and CLL (OR: 4.0, CI: 1.7-9.6, *P.value*: 0.001).

The results of many studies concerning with the association of P53 Arg/Pro polymorphism in haematological and non-haematological malignancies in different populations showed conflict results. Dunna *et al.,* 2012 studied the association of P53 codon 72 polymorphism with acute leukemia and reported that Arg/Pro genotype was the most common in both patients and controls, also they didn't found a significant correlation between Arg/Pro genotype and ALL. Another study by Sturml *et al*., 2005 reported that in patients with B-CLL Arg/Arg was the most common followed by Arg/Pro and Pro/Pro. Molecular epidemiological studies conducted on patients with non-haematological malignancies showed that Pro/Pro genotype has been associated with increased susceptibility to stomach cancer and lung cancer (Yi and Lee, 2006; Wang *et al*., 1999). However presence of Arg/Arg genotype has been associated with increased susceptibility to cervical cancer and ovarian cancer (Agorastos *et al*., 2004; Li1 *et al*., 2002). Presence of Arg/Pro genotype has been associated with increased susceptibility to breast cancer (Boroujeni1 *et al*., 2012). On the other hand the association of the P53 genotypes with susceptibility to oral cancer was not observed. These variations in these studies could be related to the ethnic variations as these studies included patients from different populations (Omori *et al*., 2004; Tandle1 *et al*., 2001). In the present study, arg allele frequency was 0.56 in patients with ALL, 0.60 in patients with CLL, and 0.88 in control group. Pro allele frequency was 0.44 in patients with ALL, 0.40 in patients with CLL, and 0.12 in control group. These findings consist with the fact that, the arginine (Arg 72) allele increases the ability of P53 to locate to mitochondria and induce cellular death, whereas proline allele (Pro 72) exhibits a lower apoptotic potential and an increased cellular arrest in G1 of the cell cycle (Bergamaschi *et al*., 2004). This more supported by our finding that, a deviation from Hardy- Weinberg equilibrium was observed in patients with ALL. Studies carried out in Sudan concluded that P53 Arg/Pro polymorphism has different pattern of frequency in different types of cancer among Sudanese patients (Eltahir *et al*., 2012).

REFERENCES

Agorastos T, Masouridou S, Lambropoulos AF, Chrisafi S, Miliaras D, Pantazis K, Constantinides TC, Kotsis A, Bontis I. 2004. P53 codon 72 polymorphism and correlation with ovarian and endometrial cancer in Greek women. *Eur. J. Cancer Prev*.13:277–280

Bergamaschi G, Merante S, Orlandi E, Galli A, Bernasconi P, Cazzola M. 2004. TP53 codon 72 polymorphism in patients with chronic myeloid leukemia. *Haematologica*., 89(7):868-869.

Boroujeni1 H.R, Karimi M, Moshkelani S and Parsaei P. 2013. Association of the p53 codon 72 polymorphism with breast cancer in central part of Iran, *African Journal of Pharmacy and Pharmacology*., 7(7):356-359.

Chen RH, Chang CT, Wang TY, Huang WL, Tsai CH, Tsai FJ. 2008. P53 Codon 72 Proline/Arginine Polymorphism and Autoimmune Thyroid Diseases, *J. Clin. Lab. Anal*. 22:321–326.

Donehower, L.A., Bardley A. 1993. The tumor suppressor p53. *Biochimicaet Biophysica Act*., 1155: 181-205.

Dumont P, Leu JI, Della Pietra AC, George DL, Murphy M. 2003. The Codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat Genet*., 33(3):357-365.

Dunna N.R, Vure S, Sailaja K, Surekha D, Raghunadharao D, Rajappa S, Vishnupriya S. 2012. TP53 Codon 72 Polymorphism and Risk of Acute Leukemia *Asian Pacific J Cancer Prev.,* 13: 347-350

Eltahir HA, Adam AA, Yahia ZA, Ali NF, Mursi DM, Higazi AM, Eid NA, Elhassan AM, Mohammed HS, Ibrahim ME. 2012. p53 Codon 72 arginine/proline polymorphism and cancer in Sudan. *Molecular Biology Reports*., 39 (12):10833-10836.

Gottlieb E, Moshe O. 1998. P53 facilitates pRb cleavage in IL-3-deprived cells: novel pro-apoptotic activity of p53. *EMBO J*., 17: 3587-96.

He XF, Su J, Zhang Y, Huang X, Liu Y, Ding DP, Wang W, Arparkorn K. 2011. Association between the p53 polymorphisms and breast cancer risk: meta-analysis based on case-control study. *Breast Cancer Res and Treat*., 130 (2):517-529.

Jiang P, Liu J, Li W, Zeng X, Tang J. 2010. Role of p53 and p21 polymorphisms in the risk of cervical cancer among Chinese women. *Acta Biochim. Biophys. Sin*., 42:671–676.

Ko LJ, Prives C .1996. p53: puzzle and paradigm. *Genes Dev*., 10:1054-72.

Lane DP, Benchimol S. 1990. p53: oncogene or anti-oncogene. *Genes & Development*., 4: 1-8.

Marin MC, Jost CA, Brooks LA, Irwin MS, O´Nicons J, Tidy JA, James N, McGregor JM, Harwood CA, Yulug IG, Vousden KH, Allday MJ, Gusterson B, Ikawa S, Hinds PW, Crook T, Kaelin WGJ. 2000. A common polymorphism acts as an intragenic modifier of mutant p53 behavior. *Nat Genet*., 25:47-54

Olivier M, Eeles R, Hollstein M, et al. 2002. The IARC TP53 database: New online mutation analysis and recommendations to users. Hum Muta., 19(6): 607-14.

Omori S, Yoshida S, Kennedy S, Negoro K, Hamana S, Barlow D, Maruo T. 2004. Polymorphism at codon 72 of the p53 gene is not associated with endometriosis in a Japanese population. *Gynecol. Investing*., 11: 232–236.

Siddique MM, Balram C, Fiszer-Maliszewska L, *et al.,* 2005. Evidence for selective expression of the p53 codon 72 polymorphs: implications in cancer development. *Cancer Epidemiol Biomarkers Prev*., 14(9):2245-52.

Sturm1 I, Bosanquet A.G, Hummel M, Dörken B and Daniel P.T. 2005. In B-CLL, the codon 72 polymorphic variants of p53 are not related to drug resistance and disease prognosis. *BMC Cancer.,* 2005., 5:1-5.

Tandle1 AT, Sanghvi V and Saranath1 D.2001. Determination of p53 genotypes in oral cancer patients from *India British Journal of Cancer*., 84(6): 739–742.

Tao Li1, Zhe-Ming Lu1, Mei Guo, M.S, Qin-Jiao Wu, M.S, Ke-Neng Chen, M.D, Hai-Ping Xing, B.S, Qiang Mei, B.S, Yang Ke, M.D., Ph.D. 2002.p53 Codon 72 Polymorphism (C/G) and the Risk of Human Papillomavirus-Associated Carcinomas in China. *CANCER*.., 95: 12.

Teodoro J.G, Sara K, Evans, *et al*. 2007. Inhibition of tumor angiogenesis by p53: a new role for the guardian of the genome: *J Mol Med*., 85: 1175-86.

Wang Y.C, Chen C.Y, Chen S.K.1999. Prognosis Patients: Association with Lung Cancer Susceptibility and p53Codon 72 Polymorphism in Taiwanese Lung Cancer. *Clin Cancer Res*., 5: 129–134.

Wang YC, Lee HS, Chen Sk, *et al*. 1999. Prognostic significance of p53 in growth control and neoplasia. *Eur J Cancer*., 35: 226-30.

Weng1, Y., Lu, L., Yuan, G., Jing G., Zhang, Z., Xie1, X., Chen, G., Zhang, J. 2012. P53 codon 72 polymorphism and Hematological Cancer Risk: An Update Meta-Analysis. PLOS ONE., 7(9):1-7.

Yi SY, Lee WJ. A p53 genetic polymorphism of gastric cancer.2006. Difference between early gastric cancer and advanced gastric cancer. *World J Gastroenterol*., 12(40): 6536-6539

Zhang, Z.-W., Newcomb, P., Hollowood, A., Feakins, R., Moorghen, M., Storey, A., Farthing, M.J.G., Alderson, D. and Holly, J. 2003. Age associated increase of codon 72 arginine p53 frequency in gastric cardia and non-cardia adenocarcinoma. *Clin Cancer Res*., 9:2151–2156

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