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ORIGINAL ARTICLE

Genetic Polymorphisms of *GPX1* (rs1050450) and *SOD1* (rs2070424) and the Risk of Liver Acute Rejection

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Abstract

Background/Aim: Transplantation is the only option for people with end-stage liver disease. With respect to improvement of this therapeutic process, rejection occurs in 50-82 % of transplant recipients. Transplanted liver is prone to exposure acute rejection because of different reasons. Oxidative stress with negative effect on the survival and function of transplanted tissue can cause graft rejection. Glutathione peroxidase 1 (GPX1) and superoxide dismutase 1 (SOD1) are known as antioxidant enzymes against ROS. The genetic factors can be disrupted clinical effects of the liver transplant. The aim of the research was to investigate the relationship between *GPX1*pro198leu and *SOD1*A251G polymorphisms with liver acute rejection risk in Iranian population.
Methods: The genotyping of *GPX1* (rs1050450) and *SOD1* (rs2070424) were performed by PCR-RFLP method in 248 liver transplanted recipients as well as 253 controls. Data were analysed by SPSS statistical software.
Results: The results of recipients following indicated that 58 patients experienced liver acute rejection. Analysis of the genotype in *GPX1*pro198leu and *SOD1*A251G polymorphisms between cases and controls showed no significant difference (*GPX1*pro198leu: OR = 1.11, 95 % CI = 0.84-1.46, p = 0.448 and *SOD1*A251G: OR = 1.17, 95 % CI = 0.72-1.92, p = 0.522). Moreover, result showed that genotype of these genes was not associated with incidence of acute rejection in patients with and without liver acute rejection (*GPX1*pro198leu: OR = 0.93, 95 % CI = 0.59-1.47, p = 0.749 and *SOD1*A251G: OR = 1.87, 95 % CI = 0.92-3.81, p = 0.083).
Conclusions: *GPX1*pro198leu and *SOD1*A251G polymorphisms does not influence the liver diseases pathogenesis and acute rejection development in liver transplant patients. Therefore, more research is needed into the genetic factors involved in transplant recipients.

Key words: Liver transplantation; Graft rejection, acute; Oxidative stress; Glutathione peroxidase *GPX1*, Superoxide dismutase (*SOD1*).

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Introduction

Transplantation is the only option for people with end-stage liver disease.[1] Transplanted cells or tissues from one person who is genetically non-identical to another resulted in 50-82 % of transplant rejection in response to the recipient's immune system. With respect to immunosuppressive drugs development, allograft acute rejection also has higher frequency compared with the other

types of rejection, its occurrence is estimated about 20-40 %. Therefore, the identification of risks factors related to rejection episodes may be useful in improving the long-term survival of the allograft and function.[2, 3]

On the other side, reactive oxygen species (ROS) are produced in different physiological and pathological processes. Due to several factors such as chronic liver disease, ischaemia-reperfusion injury, anaemia, infection, blood vessels injury and immunosuppressive drugs, the imbalance of oxidative state in liver transplant recipients after transplantation is increased. If antioxidant enzymes unable to adequately response to decrease free radicals, the release of ROS is associated with damage to vital biomolecules, resulting in tissue injury and organ dysfunction.[4] For allograft function in recipients, it is therefore essential to try to reduce or eliminate the effects of free radicals.[5]

The main components of cellular defence against oxidative stress include glutathione peroxidase and superoxide dismutase in humans. Previous studies suggested that changes in the amount of these enzymes caused oxidative stress and complications such as graft rejection in recipients.[6] Moreover, the genetic variations can be disrupted clinical effects of the liver transplant.[7] The diagnosis of candidate genes is therefore important, as these genes can affect liver function following transplantation. Glutathione peroxidase 1 (GPX1) is expressed in humans and its functions is defence of cell against oxidative stress by reducing H_2O_2 to H_2O. The gene encoding GPX1 is located on chromosome 3 (3p21.3) at the exon 2, with length of 1.424 kb.[8, 9] *SOD1* is located on chromosome 21(21q22.1) at intron 3, with length of 9.309 kb. Superoxide dismutase 1 (SOD1) is a homodimeric protein dependent on copper and zinc. SOD1 plays a major role with superoxide conversion to oxygen and hydrogen peroxide, thereby limits oxidative products.[10, 11] It should be noted that previous studies have shown a significant association between *GPX1* pro198leu polymorphisms and some diseases such as diabetes,[12] breast cancer,[13] bladder cancer[14] and Alzheimer's disease.[15] Moreover, studies focused on *SOD1*A251G polymorphism suggested that this polymorphism is associated with many diseases and cancers such as gastric cancer,[16] age-related cataract,[17] Alzheimer's[18] and NIHL.[1, 19] Identification of risk factors related to rejection episodes may improve long-term allograft survival. Since, GPX1 and SOD1 are important regulators involved in reducing oxidative stress. Thus, the aim of the research was to investigate the relationship between *GPX1*pro198leu and *SOD1*A251G polymorphisms with liver acute rejection risk in Iranian population.

Methods

In this study 248 samples of liver transplant patients (226 blood samples/22 Buffy coats) who had transplant between 2011-2015 from Transplantation Centre of Namazi Hospital (Shiraz, southern Iran) and 253 healthy individuals from Shiraz population were collected. The donor types were including 223 cadaver samples and 25 unrelated alive samples. Patients were followed up for at least 6 months to recording acute rejection according to Banff criteria.[20] Medical information was collected from patients' medical report. In this scheme, the mean age of liver transplant recipients and samples were 32.9 ±19.3 and 33.8 ±11.0 years respectively. The control group was age- and gender-matched to the patients.

DNA extraction and genotyping

A standard protocol was used to extract genomic DNA from blood samples from cases and controls.[21] *GPX1*pro198leu and *SOD1*A251G primers for this polymerase chain reaction (PCR) were the following (Table 1) and genotypes were determined as previously described.[14, 17]

Statistical analysis

The Chi-square test was used to *GPX1*pro198leu and *SOD1*A251G polymorphisms to define Hardy-Weinberg equilibrium in control group. The associations between the polymorphisms' genotypes of *GPX1*pro198leu and *SOD1*A251G with liv-

Table 1: Primers and restriction enzymes used for polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

Primer name	Primer sequence	Annealing temperature (°C)	Restriction enzyme/Allele/size	Product size (bp)
GPX1(F)	5′-AAGGTGTTCCTCCCTCGTAGGT-3′	60	*ApaI* C-74-117 T-191	191 117 74
GPX1(R)	5′-CTACGCAGGTACAGCCGCCGCT-3′			
SOD1(F)	5′-AGTACTGTCAACCACTAGC-3′	54	*MspI* A-327 G-126-201	327 201 126
SOD1(R)	5′-ACAGCTCTTCAAACAAGGC-3′			

er diseases development and liver acute rejection risk were assessed by calculating OR and 95 % CI and analysed by logistic regression analysis by SPSS software. Statistical significance was considered with the probability of $p < 0.05$.

Results

The genotypes frequencies for *GPX1*pro198leu and *SOD1*A251G polymorphisms in control group were Hardy Weinberg equilibrium (for *GPX1*pro-198leu: $\chi^2 = 1.72$, $df = 1$, $p > 0.05$, for *SOD1*A251G: $\chi^2 = 0.001$, $df = 1$, $p > 0.05$). Logistic regression analysis was used to investigate the genotypes and allele frequencies of *SOD1*A251G and *GPX-1*pro198leu polymorphisms between control and liver diseases groups (Table 2).

Table 2: Distribution of GPX1pro198leu and SOD1A251G polymorphisms in cases and controls with risk of liver disease

Polymorphism	Controls (%)	Cases (%)	OR	95 % CI	p-value
***GPX1*pro198leu**					
CC	120 (48.8)	129 (51.0)	1.00	-	-
CT	109 (44.0)	109 (43.1)	1.07	0.75-1.55	0.697
TT	19 (7.7)	15 (5.9)	1.37	0.66-2.88	0.401
CT + TT	128 (51.7)	124 (49.0)	1.11	0.78-1.57	0.562
Alleles					
C	349 (70.4)	367 (72.5)	1.00	-	-
T	147 (26.9)	139 (27.5)	1.11	0.84-1.46	0.448
***SOD1*A251G**					
AA	218 (87.6)	213 (85.9)	1.00	-	-
AG	30 (12.0)	33 (13.3)	1.13	0.66-1.91	0.661
GG	1 (0.4)	2 (0.8)	2.05	0.18-22.74	0.560
AG+GG	31 (12.4)	35 (14.1)	1.16	0.69-1.94	0.585
Alleles					
A	466 (93.6)	459 (92.5)	1.00	-	-
G	32 (6.4)	37 (7.5)	1.17	0.72-1.92	0.522

OR: odds ratio; CI: confidence interval;

Table 3: Distribution of GPX1pro198leu and SOD1A251G polymorphisms in patients with acute rejection (AR) and non-acute rejection (non-AR)

Polymorphism	Non-AR (%)	AR (%)	OR	95 % CI	p-value
*GPX1*pro198leu					
CC	90 (47.4)	30 (51.7)	1.00	-	-
CT	86 (45.3)	23 (39.7)	0.80	0.43-1.49	0.485
TT	14 (7.4)	5 (8.6)	1.07	0.36-3.22	0.902
CT + TT	100 (61.7)	28 (48.3)	0.78	0.43-1.41	0.418
Alleles					
C	266 (70.0)	83 (71.6)	1.00	-	-
T	114 (30.0)	33 (28.6)	0.93	0.59-1.47	0.749
*SOD1*A251G					
AA	166 (87.4)	47 (81.1)	1.00	-	-
AG	24 (12.6)	9 (15.5)	1.32	0.58-3.04	
GG	0 (0.0)	2 (3.4)	-	-	-
AG+GG	24 (12.6)	11 (18.9)	1.62	0.74-3.54	0.228
Alleles					
A	356 (93.7)	103 (88.8)	1.00	-	-
G	24 (6.3)	13 (11.2)	1.87	0.92-3.81	0.083

OR: odds ratio; CI: confidence interval;

Table 4: Coincidence effect of GPX1pro198leu and SOD1A251G polymorphisms in cases and controls

SOD1	*GPX1*	Control	Case	OR	95 % CI	p-value
AA	CT+TT	109	109	1.00	-	-
AA	CC	109	104	0.95	0.65-1.39	0.807
AG+GG	CT+TT	14	19	1.36	0.65-2.84	0.418
AG+GG	CC	17	16	0.94	0.45-1.96	0.871

OR: odds ratio; CI: confidence interval;

Table 5: Coincidence effect of GPX1pro198leu and SOD1A251G polymorphisms in patients with acute rejection (AR) and non-acute rejection (non-AR)

SOD1	*GPX1*	Non-AR	AR	OR	95 % CI	p-value
AA	CT+TT	87	22	1.00	-	-
AA	CC	79	25	1.25	0.65-2.39	0.498
AG+GG	CT+TT	13	6	1.82	0.62-5.34	0.272
AG+GG	CC	11	5	1.80	0.57-5.71	0.320

OR: odds ratio; CI: confidence interval;

There was not a significant association between *SOD1*A251G and *GPX1*pro198leu polymorphisms with development of liver diseases (*GPX1*pro-198leu OR = 1.11, p = 0.448 and *SOD1*A251G: OR = 1.17, p = 0.522).

Moreover, after 6 months, the biopsy results among 248 liver transplant recipients indicated that 58 recipients (23.4 %) experienced acute rejection. The genotypes and allele frequencies *GPX1*pro198leu and *SOD1*A251G polymorphisms between patients with acute rejection (AR) and non-acute rejection (non-AR) showed no significant difference (Table 3; *GPX1*pro198leu: OR = 0.93, p = 0.749 and *SOD1*A251G: OR = 1.87, p = 0.083). The analysis of the coincidence effect of *GPX1*pro-198leu and *SOD1*A251G polymorphisms in cases and controls also in patients with acute rejection (AR) and non-acute rejection (non-AR) showed no significant differences between groups (Table 4 and Table 5).

Discussion

One of the major factors in personal susceptibility to cancer and other diseases are genetic polymorphisms.[22] Liver is known as detoxification organ in the body. One of the main problems for graft survival after liver transplantation is acute liver rejection. In addition, previous research has suggested that genetic factors are associated with the stimulation of the immune system that leads to rejection and allograft failure. However, the main role of genetic factors is still considered to be a major problem in the transplantation process. On the other hand, the unbalance between oxidant and antioxidant precursors of tissue al-

lograft in recipients causes to the development of allograft dysfunction.[23, 24] Several studies have shown the effect of *GPX1*pro198leu polymorphism with predisposition to some diseases such as breast cancer,[13] PTDM,[25] cancerous tumors[26] and for *SOD1*A251G polymorphism such as age-related cataract,[17] breast cancer.[27] Lack of association between liver acute rejection with *GSTT1*, *GSTT2* and *CTLA4 CT60 A/G* polymorphisms was observed.[5, 28] GPX activity was influenced by the *GPX1*pro198leu genotype. The activity of SOD1 due to the *SOD1*A251G polymorphism is not available.[29] A previous paper published by researchers showed that there was no association between the *GPX1*pro198leu and *SOD1*A251G polymorphisms and the risk of acute kidney rejection.[30]

It should be added that the organ rejection is immunological process that determined by the complex of various genes combination. Nickerson and associates suggested that differences in genetic background may account for inter-individual alloimmune responses in transplant recipients.[31] Perhaps, one of the reasons that we cannot observe any significant relation is overlap function with other members of family enzymes. The two main limitations of current study were the limited number of samples and the fact that only single polymorphism for the *GPX1* and *SOD1* genes were searched. There are several other single nucleotide polymorphisms for these genes in humans. These were not investigated in this study.

Conclusion

Present study showed no significant difference between *GPX1*pro198leu and *SOD1*A251G polymorphisms and liver acute rejection or liver diseases risk. As a result, *GPX1*pro198leu and *SOD1*A251G polymorphisms does not influence in the liver diseases pathogenesis and acute rejection development in liver transplant patients. Organ rejection is immunological process that determined by the complex of various genes combination. Trials with more patients followed for longer and more detailed data on donors may help understanding the effects of involved factors in acute rejection process after liver transplantation.

Ethics

The study was approved by the Ethics Committee of the Biology Department, University of Shiraz, decision No ECBD-SU-13931006), dated 4 March 2018.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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References

1. Paloyo SR, David-Paloyo FP, Liquete RMR, Ona ET. Liver Transplantation in the Philippines: light at the end of the tunnel. Transplant Proc. 2024 Dec;56(10):2178-82. doi: 10.1016/j.transproceed.2024.11.009.
2. Abbas A, Lichman AH, Pillai S. Cellular and molecular immunology. 10th edition. Chapter17th. New South Wales: Saunders 2021; pp: 762-810.
3. Ronca V, Wootton G, Milani C, Cain O. The immunological basis of liver allograft rejection. Front Immunol. 2020 Sep 2;11:2155. doi: 10.3389/fimmu.2020.02155.
4. Czubkowski P, Socha P, Pawłowska J. Studies on hepatic disorders. Chapter 17. Berlin: Springer 2015; pp: 371-388.
5. Azarpira N, Nikeghbalian S, Geramizadeh B, Darai M. Influence of glutathione S-transferase M1 and T1 polymorphisms with acute rejection in Iranian liver transplant recipients. Mol Biol Rep. 2010 Jan;37(1):21-5. doi: 10.1007/s11033-009-9487-5.
6. Lubos E, Loscalzo J, Handy DE. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal. 2011;15(7):1957-97. doi: 10.1089/ars.2010.3586.
7. 7.Azarpira N, Namazi S, Malahi S, Kazemi K. Association of endothelial nitric oxide synthase gene polymorphisms with acute rejection in liver transplant recipients. Exp Clin Transplant. 2016;14(3):307-12. doi: 10.6002/ect.2014.0037.
8. Brigelius-Flohé R, Maiorino M. Glutathione peroxidases. Biochimica et Biophysica Acta. 2013;1830(5):3289–303. doi: 10.1016/j.bbagen.2012.11.020.
9. Ishida K, Morino T, Takagi K, Sukenaga Y. Nucleotide sequence of a human gene for glutathione peroxidase. Nucleic Acids Res. 1987;15(23):10051. doi: 10.1093/nar/15.23.10051.
10. Kase BA, Northrup H, Morrison AC, Davidson CM, Goiffon AM, Fletcher JM, et al. Association of copper-zinc superoxide dismutase (SOD1) and manganese superoxide dismutase (SOD2) genes with nonsyndromic myelomeningocele. Birth Defects Res A Clin Mol Teratol. 2012 Oct;94(10):762-9. doi: 10.1002/bdra.23065. Epub 2012 Sep 13. Erratum in: Birth Defects Res A Clin Mol Teratol. 2013 Jan;97(1):68.
11. Estévez AG, Franco M, Dennys CN, Rossi F. Current advances in amyotrophic lateral sclerosis. Chapter 5th. London: InTech 2013; pp. 141-158.
12. Vats P, Sagar N, Singh N, Banerjee M. Association of superoxide dismutases (SOD1 and SOD2) and glutathione peroxidase 1 (GPx1) gene polymorphisms with Type 2 diabetes mellitus. Free Radic Res. 2015;49(1):17-24. doi: 10.3109/10715762.2014.971782.
13. Ravn-Haren G, Olsen A, Tjønneland A, Dragsted LO, Nexø BA, Wallin H, et al. Associations between GPX1 Pro198Leu polymorphism, erythrocyte GPX activity, alcohol consumption and breast cancer risk in a prospective cohort study. Carcinogenesis. 2006 Apr;27(4):820-5. doi: 10.1093/carcin/bgi267.
14. Paz-y-Miño C, Muñoz MJ, López-Cortés A, Cabrera A, Palacios A, Castro B, et al. Frequency of polymorphisms pro198leu in GPX-1 gene and ile58thr in Mn-SOD gene in the altitude Ecuadorian population with bladder cancer. Oncol Res. 2010;18(8):395-400. doi: 10.3727/096504010x12644422320780.
15. Paz-y-Miño C, Carrera C, López-Cortés A, Muñoz MJ, Cumbal N, Castro B, et al. Genetic polymorphisms in apolipoprotein E and glutathione peroxidase 1 genes in the Ecuadorian population affected with Alzheimer's disease. Am J Med Sci. 2010 Nov;340(5):373-7. doi: 10.1097/MAJ.0b013e3181e93475.
16. Ebrahimpour S, Saadat I. Association of CAT C-262T and SOD1 A251G single nucleotide polymorphisms susceptible to gastric cancer. Mol Biol Res Commun. 2014;3(4):223-29. PMID: 27843986.
17. Zhang Y, Zhang L, Sun D, Li Z, Wang L, Liu P. Genetic polymorphisms of superoxide dismutases, catalase, and glutathione peroxidase in age-related cataract. Mol Vis. 2011;17:2325-32. PMID: 21921984.
18. Spisak K, Klimkowicz-Mrowiec A, Pera J, Dziedzic T, Aleksandra G, Slowik A. rs2070424 of the SOD1 gene is associated with risk of Alzheimer's disease. Neurol Neurochir Pol. 2014;48(5):342-5. doi: 10.1016/j.pjnns.2014.09.002.
19. Liu YM, Li XD, Guo X, Liu B, Lin AH, Rao SQ. Association between polymorphisms in SOD1 and noise-induced hearing loss in Chinese workers. Acta Otolaryngol. 2010 Apr;130(4):477-86. doi: 10.3109/00016480903253587.
20. Banff Working Group; Demetris AJ, Adeyi O, Bellamy CO, Clouston A, Charlotte F, Czaja A, et al. Liver biopsy interpretation for causes of late liver allograft dysfunction. Hepatology. 2006 Aug;44(2):489-501. doi: 10.1002/hep.21280.
21. Newton CR. Mutational analysis: known mutations: In: Mcpherson MJ, Hames D, Talor GR, editors. PCR2. A parcetical Approach. Oxford: IRL Press 1995; pp. 219-222.
22. Suzen HS, Gucyener E, Sakalli O, Uckun Z, Kose G, Ustel D, et al. CAT C-262T and GPX1 Pro198Leu polymorphisms in a Turkish population. Mol Biol Rep. 2010 Jan;37(1):87-92. doi: 10.1007/s11033-009-9540-4. Epub 2009 May 8. Erratum in: Mol Biol Rep. 2011 Jan;38(1):685.

23. Taub R. Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol. 2004;5(10): 836-47. doi: 10.1038/nrm1489.
24. Va´zquez-Medina JP, Zenteno-Savı´n T, Elsner R. Antioxidant enzymes in ringed seal tissues: potential protection against dive-associated ischemia/reperfusion. Comp Biochem Physiol C Toxicol Pharmacol. 2006;142(3-4):198–204. doi: 10.1016/j.cbpc.2005.09.004.
25. Dutkiewicz G, Domanski L, Pawlik A, Binczak-Kuleta A, Safranow K, Ciechanowicz A, et al. Polymorphisms of superoxide dismutase, glutathione peroxidase and catalase genes in patients with post-transplant diabetes mellitus. Arch Med Res. 2010 Jul;41(5):350-5. doi: 10.1016/j.arcmed.2010.06.006.
26. Chen J, Cao Q, Qin C, Shao P, Wu Y, Wang M, et al. GPx-1 polymorphism (rs1050450) contributes to tumor susceptibility: evidence from meta-analysis. J Cancer Res Clin Oncol. 2011 Oct;137(10):1553-61. doi: 10.1007/s00432-011-1033-x.
27. Oestergaard MZ, Tyrer J, Cebrian A, Shah M, Dunning AM, Ponder BA, et al. Interactions between genes involved in the antioxidant defence system and breast cancer risk. Br J Cancer. 2006 Aug 21;95(4):525-31. doi: 10.1038/sj.bjc.6603272.
28. Azarpira N, Malekhosseini A, Hosein Aghdaie M, Daraie M. CTLA4 CT60 A/G Gene Polymorphism in Liver Transplant Recipients. Exp Clin Transplant. 2010;8(3):210-213. PMID: 20716038.
29. Hansen RD, Krath BN, Frederiksen K, Tjønneland A, Overvad K, Roswall N, et al. GPX1 Pro(198)Leu polymorphism, erythrocyte GPX activity, interaction with alcohol consumption and smoking, and risk of colorectal cancer. Mutat Res. 2009 May 12;664(1-2):13-9. doi: 10.1016/j.mrfmmm.2009.01.009.
30. Rayatpisheh S, Karimi MH, Saadat I. Association of GPX1 Pro198Leu and SOD1 A251G polymorphisms with risk of acute kidney rejection. Scr Med. 2025 Jan-Feb;56(1):99-104. doi:10.5937/scriptamed56-55643.
31. Nickerson P. The impact of immune gene polymorphisms in kidney and liver transplantation. Clin Lab Med. 2008;28(3):455-468. doi: 10.1016/j.cll.2008.09.003.