

Evaluation of Gene Expression Pattern of IL1B and IL10 Cytokines Following Vitamin C Administration among Brain-Dead Liver Donors

Jafar Mehrabi Sisakht,^{1,2} Mohammad Bagher Khosravi,³ Soheila Milani,³ Masoumeh Kazemi,² and

Seyed Mohammad Bagher Tabei^{2,4,*}

¹Student Research Committee, Shiraz University of Medical Sciences, Shiraz, IR Iran

²Departments of Medical Genetics, Shiraz University of Medical Sciences, Shiraz, IR Iran

³Anesthesiology and Critical Care Research Center, Shiraz University of Medical Sciences, Shiraz, IR Iran

⁴Maternal-Fetal Medicines Research Center, Shiraz University of Medical Sciences, Shiraz, IR Iran

*Corresponding author: Seyed Mohammad Bagher Tabei, Department of Medical Genetics, Shiraz University of Medical Sciences, 7134853185, Shiraz, IR Iran. Tel: +98-7112349610, Fax: +98-7112349610, E-mail: tabeismb@sums.ac.ir.

Received 2016 November 06; Accepted 2016 November 26.

Abstract

Background: Inflammatory events following brain death (BD) decrease the quality of donor organs which can affect the outcome of the transplantation. It experimentally and clinically is verified that the level of inflammatory cytokines is increased in the BD of the donor results. In experiments, the in vitro use of vitamin C can successfully decrease the levels of inflammatory cytokines.

Objectives: The aim of this study was to investigate the influence of vitamin C administration on the inflammatory status of the graft in BD liver donors.

Methods: This study was conducted at Nemazee Hospital's transplant center (Shiraz, Iran). In this interventional study, the BD liver donors (n = 40) were randomly divided into two groups. The control group received only the routine intensive care unit (ICU) considerations, and the intervention group was treated with Vitamin C before harvesting organ (100 mg/kg, initially followed by 100 mg/kg/6h until organ removal). Blood samples were taken from BD patients in the intervention and control groups 3 times: 6 hours before operation, immediately after laparotomy, and immediately prior to clamping the aorta to assess the gene expression ratio of Interleukin 1 beta (IL1B) and Interleukin 10 (IL10) cytokines using real time polymerase chain reaction (PCR) (n = 40). Soluble serum cytokines were measured by enzyme-linked immunosorbent assay (ELISA) (n = 24).

Results: No significant differences were observed in mRNA expression ratio of IL1B and IL10 between two groups. Despite an acceptable decrease in serum concentration of the inflammatory markers IL1B and IL10 at time point 2 minus time point 1 (T2-1), no significant differences were observed in vitamin C-treated BD donors compared with the control donors. There was a significant difference among aspartate aminotransferase (AST) and alanine aminotransferase (ALT) changes on the 3rd to 1st days after operation between the control and intervention recipients (P < 0.05).

Conclusions: The present study suggests a beneficial effect of vitamin C administration on post-transplant function of the liver from BD liver donors.

Keywords: Brain Death, Liver Transplantation, Vitamin C, IL10, IL1B

1. Background

Except the kidney, brain dead (BD) donors are the main sources of organs for organ transplantation in the United States; Asia and Western countries (1-3). Organ transplantation is often the only treatment option at end-stage organ diseases (2, 4). Evidences have shown that BD causes inflammatory changes in the donors (5-7). A study on the liver of BD donors has shown that BD associated with other factors related to the patient's clinical status leads to dysregulation of the genes in BD patients compared with the control subjects. It contains the up-regulation and down-regulation of many genes in different pathways. Up-regulated genes often include genes involved in immune and inflammatory responses, cell signaling, stress path-

ways, apoptosis, and cell adhesion (1, 8). Moreover, a systemic inflammatory response in BD donors occurs, causing further damages to the organs, e.g. the increased inflammatory cytokines such as tumour necrosis factor alpha (TNF- α), Interleukin 1 beta (IL1B), Interleukin 6 (IL6), Interleukin 10 (IL10) and macrophage inflammatory protein 1-alpha (MIP-1 α) in donor organs (4, 9). In this context, the increased cold ischemia time, unspecific inflammatory events, additional recipient, and donor-related risk factors are crucial (10).

The "Catecholamine storm" as a consequence of BD leads to ischemia which is associated with a reduced supply of oxygen and oxidative damages (11, 12). Studies have shown that these Oxidative damages can result in activation of the transcription factor NF-kB and, subsequently, an

overexpression of inflammatory proteins (13).

The hours between confirmation of BD and organ removal might provide an opportunity to diminish the deleterious effects of BD. There are some interventions that can improve the viability of BD donor liver transplants. For instance, application of methylprednisolone (14), glucocorticoids (15) and glycine (16) had a significant effect on inflammation, graft rejection, and survival.

Vitamin C (ascorbic acid) is an essential water-soluble nutrient. It may protect from dysregulation of the immune-inflammatory response by its antioxidant properties (17). Despite efforts on the in vitro use of vitamin C as a modulator of the immune system (18-20), clinical reports are still limited to analyze vitamin C application in BD liver transplantation.

2. Objectives

The goal of this study was to evaluate the effect of vitamin C treatment in BD donors and its implications on inflammatory responses and early graft function. For this purpose, we designed a randomized trial in BD liver donors.

3. Methods

3.1. Patients and Samples

This interventional study was approved by the ethics committee of Shiraz University of Medical Sciences, Shiraz, Iran and it was done from April 2012 to June 2013 at Nemazee hospital transplant center (Shiraz, Iran). Blood samples were obtained from the two groups of liver transplant donors (10 to 60 years old): intervention group (Vitamin C-treated BD, n = 20) and control group (BD, n = 20, Table 1). The first blood samples were taken 6 hours prior to transplantation surgery (time point 1). The second blood samples were collected immediately after laparotomy (time point 2), and the third one were obtained before clamping the aorta (time point 3). The intervention group received vitamin C with a primary dose of 100 mg/kg 6 hours before operation, and it immediately was continued with vitamin infusion with a dose of 100 mg/kg (per 6 hours) until the end of the surgery and organ removal. The control group received only the routine intensive care unit (ICU) considerations. The researchers who did the experiments were blinded. This study was registered in the Iranian registry of clinical trials (IRCT) (IRCT registration No.: IRCT2012061410031N1).

3.2. Total RNA Isolation and cDNA Synthesis

The total RNA was successfully extracted from fresh blood using QIAamp RNA blood mini kit (Qiagen, USA) according to the manufacturer's instructions. RNA concentration, purity, and integrity were determined using Nano Drop ND1000 spectrophotometer (Thermo Scientific) and electrophoresis of the 5 μ L extracted RNA on 2% agarose gels, stained with ethidium bromide and visualization under the ultraviolet (UV) light. The isolated RNA was stored at -70°C until further manipulations. All preparation and handling steps of RNA took place in a laminar flow hood, under RNase-free conditions.

cDNA from 500 ng of the total RNA isolated from PBMCs was synthesized in 20 μ L total volume using RevertAid™ H Minus first strand cDNA synthesis kit (Fermentase, Lithuania) according to the manufacturer's instructions and then stored at -20°C. RNA integrity of the cDNA preparations was tested by polymerase chain reaction (PCR) amplification of a 496-bp area of the glyceraldehyde-3-phosphate deshydrogenase (GAPDH) housekeeping gene.

3.3. Primer Design

Gene sequence information was obtained using nucleotide databases (<http://www.ncbi.nlm.nih.gov/gene/>). For each target, gene Primer pairs were designed using the Allele ID6 software in order to obtain amplicons ranging from 100 to 200 bp, and specifically were designed to span exon/exon boundaries and then blasted with PRIMER-BLAST (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>) to confirm the specificity of the primers set. The primers were synthesized by Bioneer Corporation (Korea). Information for primers and the selected genes is listed in Table 1.

3.4. Quantitative Real Time PCR

Real-time PCR reactions were performed in a 96-well plate format on ABI 7500 real-time PCR system (Applied Biosystems, USA). Each reaction contained 1 μ L cDNA template, 0.4 μ L of each primer (20 pmole), 0.1 μ L ROX Reference Dye (50 x), and 10 μ L of SYBR® Premix Ex Taq (2 x) (TliRNaseH Plus), Bulk (Takara, Japan) in the final volume of 20 μ L. The real-time PCR condition was followed through the manufacturer's instruction: stage 1: initial denaturation, 1 cycle at 95°C for 30 seconds followed by stage 2: PCR, 40 cycles, each cycle including 95°C for 5 seconds, 60°C for 34 seconds. Then, a melting curve was produced to confirm a single gene-specific peak and to detect primer-dimer formation by increasing the temperature to 95°C for 5 seconds, reducing to 60°C for 1 minute and the stepwise increase of temperature from 60 to 97°C at the

Table 1. Primers for Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Primer Name	Sequence (5' - 3')	Length, bp ^a	Accession No. ^b
IL10-F	GGAGGACTTTAAGGGTTAC	187	NM_000572.2
IL10-R	TTCACAGGGAAGAAATCG		
IL1B-F	TGGCTTATTACAGTGGCAATG	134	NM_000576.2
IL1B-R	GTGGTGGTCGGAGATTCG		
GAPDH-F	GCTCTCTGCTCCTCTGTTC	114	NM_002046.4
GAPDH-R	CGACCAATCCGTTGACTCC		

^aAmplicon length in base pairs.

^bGenBank accession number of Homo sapiens mRNAs. Available online at <http://www.ncbi.nlm.nih.gov/>.

ramp rate of 0.11°C/s with continuous monitoring of the fluorescence. Additionally, the amplicons' specificity and size were also evaluated by sequencing of qRT-PCR products and standard 2% agarose gel electrophoresis. For each primer pair, PCR efficiency (E) was calculated using a standard curve derived from a RT-PCR product serially diluted 10-fold over eight measuring points. Data normalization was performed using GAPDH as the housekeeping gene. The cycle threshold (Ct) values for the housekeeping gene and the genes of interest were automatically calculated for each reaction by the 7500 software v2.0.1 with default parameters. The relative expression value was calculated with Pfaffl formula $[(E \text{ target})^{\Delta C_t \text{ target (control - Sample)}}] / [(E \text{ ref})^{\Delta C_t \text{ ref (control - Sample)}}]$. The samples were considered negative when the Ct values exceeded 40 cycles.

3.5. ELISA Test

The serum levels of IL1B and IL10 cytokines were measured using the human IL1B and IL10 Platinum ELISA kit (BT Laboratory, China) according to the manufacturer's instructions. The measurements were performed for 24 samples (12 in each group).

3.6. Clinical Parameters

Blood samples were taken from every recipient of each group 3 times (1st, 3rd and 10th postoperative days) to assess the serum values of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Total-bilirubin. Measurement of this biochemical factors was performed routinely at the laboratory of Nemazee hospital, Shiraz, Iran.

3.7. Data Analysis and Statistics

Statistical analyses were performed using SPSS-15 statistical software (SPSS Inc, Chicago, USA). Differences between the two groups were analyzed by non-parametric Mann-Whitney test. Statistical significance was set at a P value < 0.05 and the results were expressed as mean ± SE.

4. Results

4.1. Donor Characteristics

There were no statistically significant differences between control and vitamin C-treated BD donors regarding age, gender, hypotension, and perioperative cardiac arrests as well as the fluids (including infusions, albumin, etc.), dopamine, and methylprednisolone applied in both groups (Table 2). The preservation time, including cold and warm ischemia time was equal in both donor groups (242 ± 83 minutes in the control vs. 238 ± 77 minutes in the vitamin C-treated BD). The time spent at the ICU was comparable in both groups (10.55 ± 3.01 hours in the control vs. 11.8 ± 4.31 hours in the Vitamin C-treated BD).

Table 2. Donor Characteristics^a

Variable	Control Group (n = 20)	Vit. C-Treated BD Group (n = 20)	P Value
Gender, % M:F	80:20:00	60:40:00	0.3
Age, y	35 ± 15.6	38 ± 15.7	0.41
Intensive care unit (ICU), h	10.55 ± 3.01	11.8 ± 4.31	0.51
Cardiac arrest, Yes/No	0/20	0/20	1
Hypotension, Yes/No	No	No	-
Infection, Yes/No	No	No	-
Cold Ischemia time, min	203.25 ± 77.8	199.75 ± 71.55	0.99
Warm Ischemia time, min	38.75 ± 5.09	38 ± 5.47	0.7
Dopamin, mg/kg/min	6 ± 2.61	6.75 ± 2.93	0.44
Methylprednisolon, mg	500	500	1

^aValues are expressed as mean ± SD.

4.2. Recipient Characteristics

No statistically significant differences were observed in patients receiving either a graft from control or Vitamin C-treated BD donors regarding age and donor gender (Table 3). The immunosuppressive regimen in both groups was based on methylprednisolone.

Table 3. Recipient Characteristics^a

Variable	Control Group (n = 20)	Vitamin C-Treated BD Group (n = 20)	P Value
Gender, % M:F	60:40	50:50	0.75
Age, y	37.45 ± 16	34.1 ± 14.2	0.46
Methylprednisolone, mg	0.97 ± 0.732	0.965 ± 0.118	0.82

^aValues are expressed as mean ± SD.

4.3. Comparison of Cytokine mRNA Expression in Peripheral Blood Samples

To evaluate the effect of vitamin C on gene expression levels of IL1B and IL10 cytokines in peripheral blood of brain-dead liver donors, we determined the changes of mRNA expression ratio of the selected genes at 3 time intervals [ratio of time point 2 to time point 1 (R2:1), Ratio of time point 3 to time point 1 (R3:1) and ratio of time point 3 to time point 2 (R3:2)] in the two groups and then compared them in the control and intervention groups. In general, our findings revealed that RNA expression ratio of IL1B was lower at R2:1 and R3:1 in the intervention group compared with the control group (P = ns, Figure 1B) and the mRNA expression ratio of IL10 was lower at R3:2 and higher at R2:1 and R3:1 in the intervention group compared with the control group (P = ns, Figure 1A). No between-group significant differences were observed in mRNA expression ratio of IL1B and IL10.

4.4. Concentration of the Soluble Cytokines in the Control and Intervention Groups

The analysis of serum concentration and its differences revealed that despite an acceptable decrease in the serum concentration of the inflammatory cytokines IL1B and IL10 at time point 2 minus time point 1 (T2-1), no significant differences were observed in vitamin C-treated BD donors compared with the control donors (TP2-1, P = ns, Figure 2A and 2B).

4.5. Follow-Up Study and Graft Function

To investigate the effect of the decreased immune activation within the graft due to donor treatment with vitamin C on the allograft outcome, aspartate aminotransferase (AST), Alanine transaminase (ALT) and total Bilirubin values were determined as the markers for liver function. The results illustrated that there was a significant difference among AST and ALT changes on the 3rd day to 1st days after operation between control and intervention recipients groups (P < 0.02 and P < 0.04, respectively, Figure 3A and 3B), so that the level of these parameters had a considerable decrease through the mentioned period. Despite such a remarkable reduction of these factors on the 3rd and 10th days, the result was not significant. Comparing the changes on the 10th to 3rd days between the two groups, no significant results were found. Regarding the serum total Bilirubin value, there was no significant difference between the two groups of recipients.

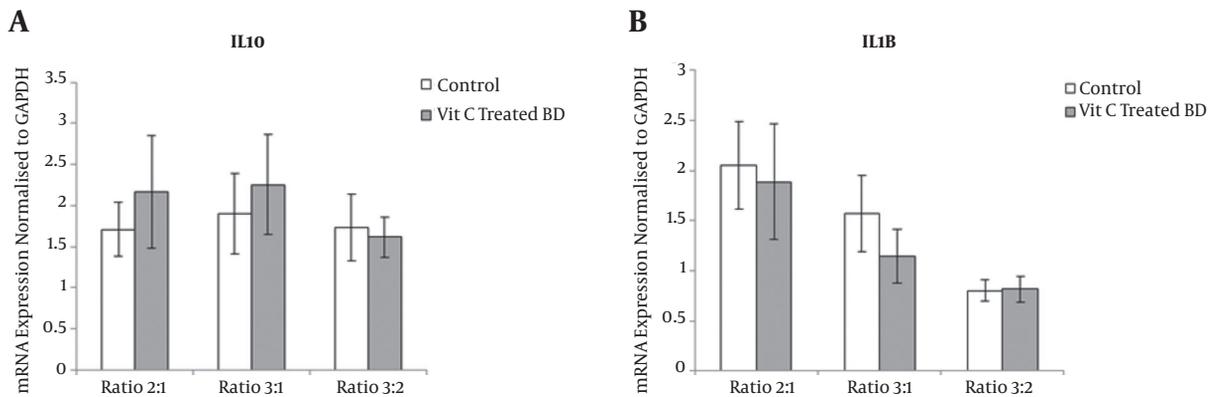
5. Discussion

This study for the first time describes the effect of in vivo vitamin C administration on inflammatory responses of the BD liver donors and graft function of the recipients. Clinical and experimental studies have shown that BD in the donors has a significant impact on the graft quality (21). This result is supported as the livers from a living donor have superior post-transplant survival rate compared with those from BD donors (22, 23). This process was associated with the increased immune activation, including the increased release and expression of pro-inflammatory as well as anti-inflammatory cytokines (4, 8).

The ischemia is a hallmark of BD (24) that subsequently promotes generation of reactive oxygen species (ROS). It in turn induces the activation of NF-KB signaling pathway and up-regulates a dozen of downstream target genes including pro-inflammatory cytokines (i.e. IL1B, TNF- α and IL 6). Therefore, antioxidant agents such as vitamin C seem to be effective in suppressing NF-KB pathway (25, 26). Accordingly, in this study, vitamin C was administrated to the BD liver donors with a primary dose of 100 mg/kg, and it was continued with the dose of 100 mg/kg/6 hours until organ removal to assess the gene expression and serum levels of IL1B and IL10 cytokines as well as a follow-up study of the recipients.

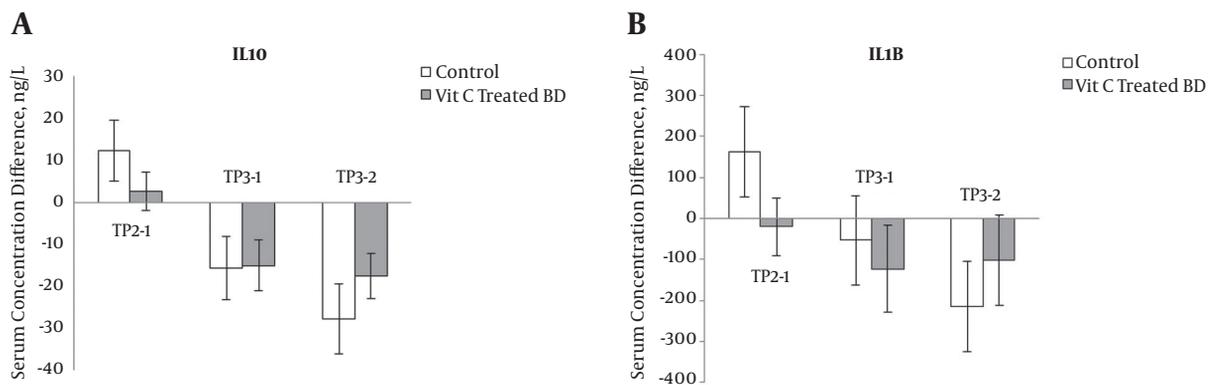
Olinga et al. and Kuecuk et al. reported that through the real-time reverse transcriptase-polymerase chain reaction (QRT-PCR) analysis of the liver tissues from BD donors, the expression levels of IL1B and IL10 mRNA before the incision were significantly higher than those of living donors (27, 28). Our results revealed that mRNA expression levels of the studied cytokines were up-regulated at all the

Figure 1. The Changes of mRNA Expression Ratio of the Inflammatory Cytokines Interleukin 10 (IL10) and Interleukin 1 Beta (IL1B) in Control and Vitamin C-Treated Brain Death (BD) Donors at 3 Time Intervals, R2:1, R3:1 and R3:2 Measured by Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR)



No between-group significant differences were observed in mRNA expression ratio of IL1B and IL10 by non-parametric Mann-Whitney test.

Figure 2. The Serum Concentration of the Inflammatory Cytokines IL1B and IL10 at T2-1 (Time Point 2 Minus Time Point 1), T3-1 and T3-2



No significant differences were observed in the vitamin C-treated BD donors compared with the control donors.

time points in two groups. Previous studies have shown that the majority of the investigated cytokines were obviously up-regulated after laparotomy of the BD donor (28-30). These observations are consistent with the results of this study indicating that gene expression and serum levels of IL1B and IL10 cytokines were more up-regulated in the peripheral blood of the two groups immediately after laparotomy. These changes are expected to increase the graft immunogenicity prior to transplantation and cause a certain decrease in the quality of donor livers.

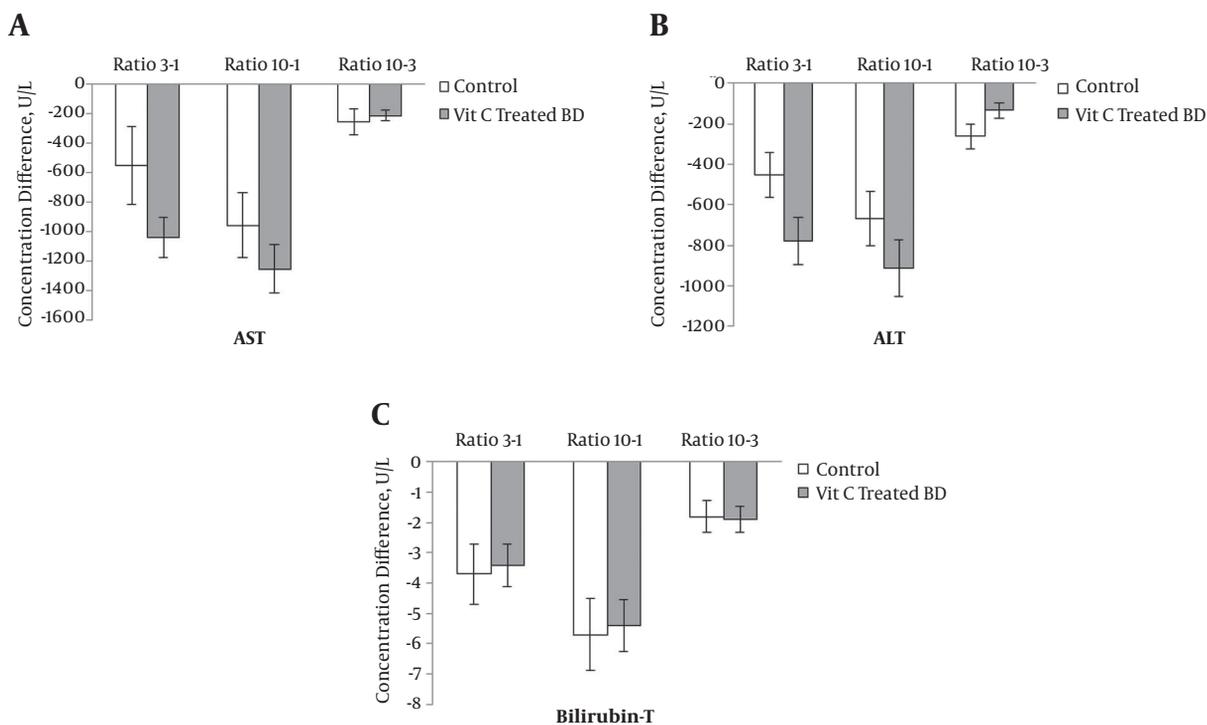
We also found that serum concentration of the inflammatory markers IL1B and IL10 as well as mRNA expression ratio of IL1B were slightly decreased in the intervention group compared with the control group at laparotomy time point. These observations suggest that vitamin C is mostly effective during laparotomy, probably due to the

high oxidative stress during laparotomy than other times.

In 2007, Weiss reported that the levels of ALT and AST in the livers of BD donors were significantly higher on the first and third days after transplantation compared with those of living donors (9). As a result, our findings revealed that vitamin C has beneficial effects on post-transplant function of the liver from BD donors, since it significantly decreases the levels of ALT and AST during the 1st to 3rd days after operation in the intervention recipients compared with the control recipients.

In fact, this study has limitations. For instance, BD liver donors received methylprednisolone and dopamine. It is proven that these immunosuppressive agents decrease the expression of cytokines in the serum and organs (14, 31). This indicates that these anti-inflammatory drugs possibly have interfered our intervention; therefore, this might be

Figure 3. The Changes of Biochemical Serum Parameters Including Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Total Bilirubin (Bilirubin-T) in the Recipients With Control Grafts and Vitamin C-Treated BD Grafts at POD3-1 (Postoperative Day 3 Minus Postoperative Day 1), POD10-1 and POD10-3



There was a significant difference between AST and ALT changes on the 3rd day to 1st days after operation between control and intervention recipients groups (* $P < 0.02$, ** $P < 0.04$).

the reason for the fact that vitamin C has not exerted the expected effects. In addition, the used amount of vitamin C might have been insufficient. Moreover, due to the peak of vitamin C influence at laparotomy time point, it seems better to start the intervention at an earlier time (for example at the time of confirming the brain death diagnosis or at the time of receiving consent for organ donation).

In conclusion, our data may provide a potential explanation for in vivo influence of vitamin C on post-transplant function of the liver from BD liver donors. Accordingly, further studies are needed to elucidate the protective effect of Vitamin C administration in BD liver donor transplantation, which is our next research target.

Acknowledgments

We would like to thank Nemazee hospital transplant center for their collaborations; also the authors would like to thank Dr. Nasrin Shokrpour for editorial assistance and Miss Roosta for statistical analysis at the center for development of clinical research of the Nemazee hospital. The

present article is extracted from the thesis written by Jafar Mehrabi Sisakht. The authors declare that they have no competing interests.

Footnotes

Authors' Contribution: Mohammad Bagher Tabei designed the project, contributed to the interpretation of the data and revision of the manuscript. Jafar Mehrabi Sisakht performed the experiments, contributed to the interpretation of the data and revision of the manuscript. Mohammad Bagher Khosravi and Soheila Milani diagnosed and critically followed-up the patients. Masoumeh Kazemi performed the experiments (as a colleague).

Financial Disclosure: We have no financial interests related to the used materials in the manuscript.

Funding/Support: This project was financially supported by the Shiraz University of Medical Sciences, grant No. 91-6146.

References

1. Danobeitia JS, Sperger JM, Hanson MS, Park EE, Chlebeck PJ, Roenneburg DA, et al. Early activation of the inflammatory response in the liver of brain-dead non-human primates. *J Surg Res*. 2012;**176**(2):639-48. doi: [10.1016/j.jss.2011.10.042](https://doi.org/10.1016/j.jss.2011.10.042). [PubMed: [22440934](https://pubmed.ncbi.nlm.nih.gov/22440934/)].
2. Zhang SJ, Wang T. The influence of brain death on donor liver and the potential mechanisms of protective intervention. *Front Med*. 2011;**5**(1):8-14. doi: [10.1007/s11684-011-0109-y](https://doi.org/10.1007/s11684-011-0109-y). [PubMed: [21681669](https://pubmed.ncbi.nlm.nih.gov/21681669/)].
3. Zahedi F, Fazel I, Larijani B. An overview of organ transplantation in Iran over three decades: with special focus on renal transplantation. *Iran J Publ Health*. 2009;**38**(1):138-49.
4. Skrabal CA, Thompson LO, Potapov EV, Southard RE, Joyce DL, Youker KA, et al. Organ-specific regulation of pro-inflammatory molecules in heart, lung, and kidney following brain death. *J Surg Res*. 2005;**123**(1):118-25. doi: [10.1016/j.jss.2004.07.245](https://doi.org/10.1016/j.jss.2004.07.245). [PubMed: [15652959](https://pubmed.ncbi.nlm.nih.gov/15652959/)].
5. Kusaka M, Pratschke J, Wilhelm MJ, Ziai F, Zandi-Nejad K, Mackenzie HS, et al. Activation of inflammatory mediators in rat renal isografts by donor brain death. *Transplantation*. 2000;**69**(3):405-10. [PubMed: [10706051](https://pubmed.ncbi.nlm.nih.gov/10706051/)].
6. Takada M, Nadeau KC, Hancock WW, Mackenzie HS, Shaw GD, Waaga AM, et al. Effects of explosive brain death on cytokine activation of peripheral organs in the rat. *Transplantation*. 1998;**65**(12):1533-42. [PubMed: [9665067](https://pubmed.ncbi.nlm.nih.gov/9665067/)].
7. Barklin A. Systemic inflammation in the brain-dead organ donor. *Acta Anaesthesiol Scand*. 2009;**53**(4):425-35. doi: [10.1111/j.1399-6576.2008.01879.x](https://doi.org/10.1111/j.1399-6576.2008.01879.x). [PubMed: [19226294](https://pubmed.ncbi.nlm.nih.gov/19226294/)].
8. Conti A. Gene Expression Profile in Liver Transplantation and the Influence of Gene Dysregulation Occurring in Deceased Donor Grafts. *Open Surg J*. 2011;**5**(1):1-11. doi: [10.2174/1874300501105010001](https://doi.org/10.2174/1874300501105010001).
9. Weiss S, Kotsch K, Francuski M, Reutzel-Selke A, Mantouvalou L, Klemz R, et al. Brain death activates donor organs and is associated with a worse I/R injury after liver transplantation. *Am J Transplant*. 2007;**7**(6):1584-93. doi: [10.1111/j.1600-6143.2007.01799.x](https://doi.org/10.1111/j.1600-6143.2007.01799.x). [PubMed: [17430397](https://pubmed.ncbi.nlm.nih.gov/17430397/)].
10. Colombo G, Gatti S, Turcatti F, Lonati C, Sordi A, Rossi G, et al. Alteration in the transcriptional profile of livers from brain-dead organ donors. *Transplantation*. 2006;**82**(1):69-79. doi: [10.1097/01.tp.0000225829.07208.58](https://doi.org/10.1097/01.tp.0000225829.07208.58). [PubMed: [16861944](https://pubmed.ncbi.nlm.nih.gov/16861944/)].
11. Novitzky D. Detrimental effects of brain death on the potential organ donor. *Transplant Proc*. 1997;**29**(8):3770-2. [PubMed: [9414920](https://pubmed.ncbi.nlm.nih.gov/9414920/)].
12. Power BM, Van Heerden PV. The physiological changes associated with brain death—current concepts and implications for treatment of the brain dead organ donor. *Anaesth Intensive Care*. 1995;**23**(1):26-36. [PubMed: [7778744](https://pubmed.ncbi.nlm.nih.gov/7778744/)].
13. Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. *Annu Rev Immunol*. 1994;**12**:141-79. doi: [10.1146/annurev.y12.040194.001041](https://doi.org/10.1146/annurev.y12.040194.001041). [PubMed: [8011280](https://pubmed.ncbi.nlm.nih.gov/8011280/)].
14. Miki C, Gunson BK, Buckels JA, Uchida K, Mohri Y, Kusunoki M. Methylprednisolone therapy in deceased donors reduces inflammation in the donor liver and improves outcome after liver transplantation. *Ann Surg*. 2009;**250**(3):502-3. doi: [10.1097/SLA.0b013e3181b4cbe0](https://doi.org/10.1097/SLA.0b013e3181b4cbe0). [PubMed: [19730189](https://pubmed.ncbi.nlm.nih.gov/19730189/)] author reply 503-4.
15. McLean KM, Duffy JY, Pandalai PK, Lyons JM, Bulcao CF, Wagner CJ, et al. Glucocorticoids alter the balance between pro- and anti-inflammatory mediators in the myocardium in a porcine model of brain death. *J Heart Lung Transplant*. 2007;**26**(1):78-84. doi: [10.1016/j.healun.2006.10.011](https://doi.org/10.1016/j.healun.2006.10.011). [PubMed: [17234521](https://pubmed.ncbi.nlm.nih.gov/17234521/)].
16. Zhang SJ, Shi JH, Tang Z, Wu Y, Chen S. Protective effects of glycine pretreatment on brain-death donor liver. *Hepatobiliary Pancreat Dis Int*. 2005;**4**(1):37-40. [PubMed: [15730916](https://pubmed.ncbi.nlm.nih.gov/15730916/)].
17. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. *J Am Coll Nutr*. 2003;**22**(1):18-35. [PubMed: [12569111](https://pubmed.ncbi.nlm.nih.gov/12569111/)].
18. Mahapatra SK, Chakraborty SP, Roy S. Immunomodulatory role of Ocimum gratissimum and ascorbic acid against nicotine-induced murine peritoneal macrophages in vitro. *Oxid Med Cell Longev*. 2011;**2011**:734319. doi: [10.1155/2011/734319](https://doi.org/10.1155/2011/734319). [PubMed: [22220218](https://pubmed.ncbi.nlm.nih.gov/22220218/)].
19. Tomofuji T, Ekuni D, Sanbe T, Irie K, Azuma T, Maruyama T, et al. Effects of vitamin C intake on gingival oxidative stress in rat periodontitis. *Free Radic Biol Med*. 2009;**46**(2):163-8. doi: [10.1016/j.freeradbiomed.2008.09.040](https://doi.org/10.1016/j.freeradbiomed.2008.09.040). [PubMed: [18983910](https://pubmed.ncbi.nlm.nih.gov/18983910/)].
20. Hartel C, Strunk T, Bucszy P, Schultz C. Effects of vitamin C on intracytoplasmic cytokine production in human whole blood monocytes and lymphocytes. *Cytokine*. 2004;**27**(4-5):101-6. doi: [10.1016/j.cyto.2004.02.004](https://doi.org/10.1016/j.cyto.2004.02.004). [PubMed: [15271375](https://pubmed.ncbi.nlm.nih.gov/15271375/)].
21. Compagnon P, Wang H, Lindell SL, Ametani MS, Mangino MJ, D'Alessandro AM, et al. Brain death does not affect hepatic allograft function and survival after orthotopic transplantation in a canine model. *Transplantation*. 2002;**73**(8):1218-27. [PubMed: [11981412](https://pubmed.ncbi.nlm.nih.gov/11981412/)].
22. Jassem W, Koo DD, Cerundolo L, Rela M, Heaton ND, Fuggle SV. Cadaveric versus living-donor livers: differences in inflammatory markers after transplantation. *Transplantation*. 2003;**76**(11):1599-603. doi: [10.1097/01.TP.0000100400.82135.DC](https://doi.org/10.1097/01.TP.0000100400.82135.DC). [PubMed: [14702531](https://pubmed.ncbi.nlm.nih.gov/14702531/)].
23. Nijboer WN, Schuur TA, van der Hoeven JA, Fekken S, Wiersema-Buist J, Leuvenink HG, et al. Effect of brain death on gene expression and tissue activation in human donor kidneys. *Transplantation*. 2004;**78**(7):978-86. [PubMed: [15480162](https://pubmed.ncbi.nlm.nih.gov/15480162/)].
24. Raichle ME. The pathophysiology of brain ischemia. *Ann Neurol*. 1983;**13**(1):2-10. doi: [10.1002/ana.410130103](https://doi.org/10.1002/ana.410130103). [PubMed: [6299175](https://pubmed.ncbi.nlm.nih.gov/6299175/)].
25. Siomek A. NF-kappaB signaling pathway and free radical impact. *Acta Biochim Pol*. 2012;**59**(3):323-31. [PubMed: [22855720](https://pubmed.ncbi.nlm.nih.gov/22855720/)].
26. O'Dea E, Hoffmann A. NF-kappaB signaling. *Wiley Interdiscip Rev Syst Biol Med*. 2009;**1**(1):107-15. doi: [10.1002/wsbm.30](https://doi.org/10.1002/wsbm.30). [PubMed: [20151024](https://pubmed.ncbi.nlm.nih.gov/20151024/)].
27. Olinga P, van der Hoeven JA, Merema MT, Freund RL, Ploeg RJ, Groothuis GM. The influence of brain death on liver function. *Liver Int*. 2005;**25**(1):109-16. doi: [10.1111/j.1478-3231.2005.01035.x](https://doi.org/10.1111/j.1478-3231.2005.01035.x). [PubMed: [15698407](https://pubmed.ncbi.nlm.nih.gov/15698407/)].
28. Kuecuk O, Mantouvalou L, Klemz R, Kotsch K, Volk HD, Jonas S, et al. Significant reduction of proinflammatory cytokines by treatment of the brain-dead donor. *Transplant Proc*. 2005;**37**(1):387-8. doi: [10.1016/j.transproceed.2004.12.165](https://doi.org/10.1016/j.transproceed.2004.12.165). [PubMed: [15808654](https://pubmed.ncbi.nlm.nih.gov/15808654/)].
29. Koo DD, Welsh KI, McLaren AJ, Roake JA, Morris PJ, Fuggle SV. Cadaver versus living donor kidneys: impact of donor factors on antigen induction before transplantation. *Kidney Int*. 1999;**56**(4):1551-9. doi: [10.1046/j.1523-1755.1999.00657.x](https://doi.org/10.1046/j.1523-1755.1999.00657.x). [PubMed: [10504507](https://pubmed.ncbi.nlm.nih.gov/10504507/)].
30. Kim YS, Lim CS, Kim S, Lee JS, Lee S, Kim ST, et al. Cadaveric renal allograft at the time of implantation has the similar immunological features with the rejecting allograft. *Transplantation*. 2000;**70**(7):1080-5. [PubMed: [11045646](https://pubmed.ncbi.nlm.nih.gov/11045646/)].
31. Hoeger S, Reisenbuechler A, Gottmann U, Doyon F, Braun C, Kaya Z, et al. Donor dopamine treatment in brain dead rats is associated with an improvement in renal function early after transplantation and a reduction in renal inflammation. *Transplant Int*. 2008;**21**(11):1072-80.