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Original Article

The effects of total parenteral nutrition on telomerase expression in rabbit

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Abstract

Aim: Total parenteral nutrition (TPN) is a technique which is use to give vitally mandatory substances in to the venous compartments whenever the gastrointestinal system cannot be used by the patients. Telomerase catalyzes DNA synthesis, which is necessary to maintain telomere length and stabilize the genome to allow continued cell proliferation. In this study, we explored the effects of TPN administration on telomerase reverse transcriptase (TERT) expression in various tissue and serum telomerase level.

Material and Methods: In this study a number of 42 same-aged albino, equal number of male and female, new zealand rabbits were use, divided in to three groups. Group 1 rabbits received TPN for 10 days via a central venous catheter. Group 2 received 50 mL/kg/day physiological saline via a central venous catheter. Group 3 served as the control group. The rabbits were sacrificed after 10 days, and serum telomerase levels were measured by enzyme-linked immunosorbent assay. TERT expression in gonadal, liver, jejunum, and skin tissues were determined immunohistochemically. Blood samples were obtained before and after TPN and saline administration in the TPN and serum saline groups, respectively, and at the end of the experiment in the control group.

Results: Telomerase expression in liver, gonads and serum level of TPN group was significantly higher than control and serum saline groups.

Conclusion: TPN may be a positive effect in liver and gonadal telomer kinetic. However, we think that TPN increases DNA damage throughout the body.

Keywords: Total Parenteral Nutrition; Telomerase Reverse Transcriptase; Liver; Ovary; Testis; Rabbit.

INTRODUCTION

The first person to attempt to deliver intravenous nutrition was Sir Christopher Wren, The architect of St. Paul's Cathedral in (1). Use of a central venous catheter allowed Dudrick to infuse concentrated solutions of glucose and aminoacids into patients (2). TPN is used to treat children who undergo gastrointestinal surgery to correct congenital and acquired lesions, and it reduces mortality (1). TPN is expensive, invasive, non-physiological, and associated with several adverse effects (3).

Telomerase is a ribonucleoprotein complex (4). When a cell divides, DNA polymerase does not completely replicate the telomeres, which become shorter after each division (5). The existing telomere length determines the number of possible future cell divisions. Ultimately, telomere shortening renders the cell senescent, and division ceases (6). Telomerase is a ribonucleoprotein complex (5). Telomere length is negatively correlated with smoking, oxidative stress, and aging, whereas a healthy lifestyle is associated with increased telomere length (7-9).

Telomerase is directly protects telomeres (5,10). Telomerase is active in germs cells, hematopoietic stem cell lines, basal epidermal cells, and certain other stem cells, but is not always found in differentiated somatic cells (11,12). Cancer cells have short, stable telomeres reflecting uncontrolled increases in telomerase level (6). As such, telomerase is the target of therapeutic research (13).

Telomerase reverse transcriptase (TERT) is a catalytic component of the telomerase complex and the rate-limiting enzyme in telomerase level (14). Nuclear TERT and telomerase contain RNAs that serve as templates for DNA elongation. Although telomerase regulation in cancer cells is well-understood, the effects of telomerase

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on normal biological processes are poorly studied. In healthy tissues, telomerase TERT is generally inactivated via transcriptional repression prior to birth, but it remains active in the ovary, testis, lymph nodes, and certain hyperplastic tissues (15–21).

In the present study, we measured serum telomerase level and assessed TERT expression in various tissues after the administration of TPN in rabbits by immunohistochemistry.

MATERIAL and METHODS

We used 42 same-aged albino New Zealand rabbits divided into three groups. The rabbits were provided by the Inonu University Experimental Research Center, Inonu University, Faculty of Medicine. The Faculty of Medicine Animal Ethics Committee approved the study. The rabbits were kept at 23°C with 12 h of light and 12 h of darkness per day and were weighed before and after the experiment.

Experimental groups

Three groups were established as group 1, fourteen rabbits received TPN via a central venous catheter for 10 days; group 2, fourteen rabbits received 0.09% (w/v) saline (50 mL/kg/day) via a central venous catheter for 10 days; and group 3, fourteen rabbits were not catheterized and did not receive any treatment.

All rabbits had free access to food and water. The food included 18.7% protein, 4.2% fats 10% cellulose, 12% starch, 5.5% ash, 0.36% methionine, 0.98% lysine, and 2.45% minerals (Korkuteli T.B.T.Y, Antalya, Turkey). The polyethylene catheters were 0.5 mm in diameter and 45 cm in length (Cavafix® Certo; Braun, Melsungen, Germany) and were inserted into the jugular vein using the cut-down method under ketamine-induced anesthesia (35 mg/kg, intramuscularly [IM]) (Ketalar; Parke-Davis, Ann Arbor, MI, USA) and Xilazin (5 mg/kg, IM) (Rompun; Bayer AG, Leverkusen, Germany).

TPN

TPN included 10% (w/v) lipid (Intralipid; Fresenius Kabi, Uppsala, Sweden), 6% (w/v) amino acids (Trophamine; Eczacibasi Baxter, Istanbul, Turkey), and trace elements (AddamelTM N; Fresenius Kabi) (Table 1). TPN was initiated 1 day after catheterization and continued for 8 h per day for 10 days. Caloric delivery was based on estimates of the caloric intake of the control group so that caloric delivery was essentially the same in all groups.

Table 1. Features of daily TPN formula

Ingredients	Dose	Volume (ml)	Calorie (kcal/g)	Osmolality (mOsm/L)
Amino acids 6%	4 g/kg	160	40	525
Dextrose 20%	18 g/kg	250	170	1250
Lipid 10%	2 g/kg	50	55	280
NaCl 0.9%	(3 mEq/kg)	50	-	310
KCl	(3 mEq/kg)	3	-	-
Mg	(1 mEq/kg)	1	-	-
Trace elements	-	1	-	-
Calcium	(1 mEq/kg)	1		
TPN		516	365	825

Tissue sampling

At the end of the experiment, the rabbits were sacrificed, and skin, jejunum, liver, ovary, and testis samples were collected. All tissues were cut into two transverse sections, fixed in 10% (v/v) formalin for 24 h, macroscopically sampled, subjected to routine tissue examination, and embedded in paraffin blocks. Slides (5-µm-thick sections) were prepared, deparaffinized, stained with hematoxylin and eosin (H&E), and stained immunohistochemically for TERT. The immunohistopathological evaluation was performed by two pathologists who were blinded to group status.

Blood samples

Blood samples were obtained before and after the administration of TPN and saline solution in the TPN and serum saline groups, respectively, and at the end of the experiment in the control group. Blood was collected into tubes containing ethylene diamine tetra-acetic acid for the measurement of telomerase level. The samples were stored at -80oC until the experiments were performed. On the day of the experiment, the blood samples were warmed to +23oC and centrifuged immediately at 4,000 rpm for 5 min; the plasma was collected for the serum analyses.

Histopathological and immunohistochemical (IHC) methods

Tissue samples from the skin, ileum, liver, ovaries, and testicles were fixed in 10% (w/v) buffered formalin, subjected to routine tissue evaluation, embedded in paraffin, and 5-µm-thick sections were prepared. The slides were exposed to 70°C for 1 h, deparaffinized in xylene, and stained with H&E prior to the histopathological examination. IHC staining employed polyclonal anti-TERT antibodies (Biorbyt, Midland, ON, Canada; catalog no. orb11463, 1:300 dilution). Prior to staining, the slides were placed in a peroxidase-quenching solution for 5 min, then placed in a microwave oven and heated in an EDTA solution for 20 min. For immunostaining, four drops of reagent (reaction buffer concentrate, Roche Diagnostics GMBH, Mannheim, Germany) was added to each slide, and the samples were incubated for 15 min and drained. The primary antibody solution (200 µL) was added and the

slides were stored overnight. Four drops of a solution of secondary antibody (from a universal kit) were then added to each slide, and, 1 h later, the slides were counterstained with hematoxylin.

Only nuclear or nucleolar staining was considered to reflect TERT-positivity of the skin, ileum, liver, ovary, and testis sections. IHC scoring was divided into three categories: Grade 1: no staining, Grade 2; less staining and Grade 3; strong staining, (Figure 1. A,B,C).

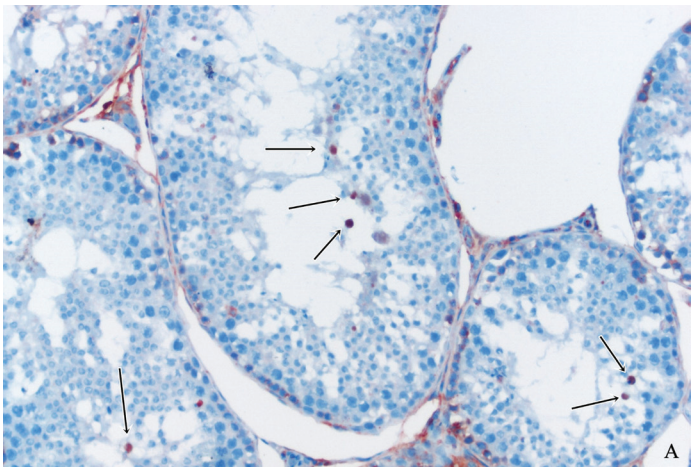


Figure 1A. Strong nuclear TERT expression in testicular spermatocytes from the TPN group. Magnification X200

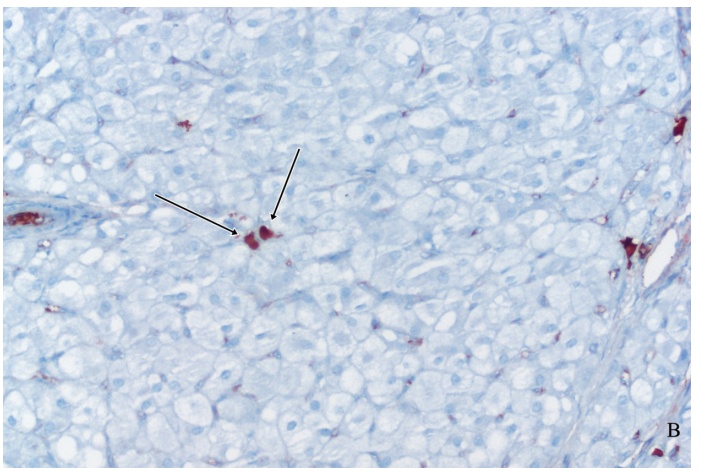


Figure 1B. Strong nuclear TERT expression in ovarian tissue from the TPN group. Magnification X200

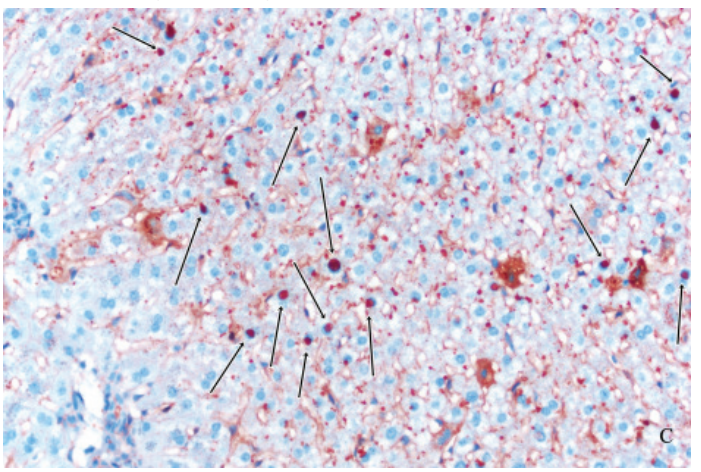


Figure 1C. Strong nuclear TERT expression in liver tissue from the TPN group. Magnification X200.

Serum telomerase level

Rabbit serum telomerase level was measured by sandwich enzyme-linked immunosorbent assay (ELISA) using a commercial Elabscience rabbit (telomerase) ELISA kit (Elabscience Biotechnology Co., Ltd., Wuhan, China) and read using a BioTek Synergy H1 reader (BioTek Instruments, Inc., Winooski, VT, USA).

Statistics

The statistical analyses were performed with SPSS version 12 (SPSS, Inc., Chicago, IL, USA). Quantitative data are presented as the mean ± standard deviation (SD), and qualitative data as percentages and counts. A one-way analysis of variance was employed to compare multiple quantitative variables.

The chi-square test was employed to determine whether the ratios of observed differences lay within the limits of the theoretical or expected ratios. Serum concentration values were assessed using the Kruskal–Wallis test. The Bonferroni-corrected Mann–Whitney U-test was used to perform multiple comparisons. A P-value <0.05 was considered to reflect significance, and a P-value <0.01 a high level of significance.

RESULTS

The rabbits in all groups weighed 2,600 ± 500 g and 2,550 ± 475 g at the beginning and the end of the experiment, respectively; the difference was not significant (P > 0.05).

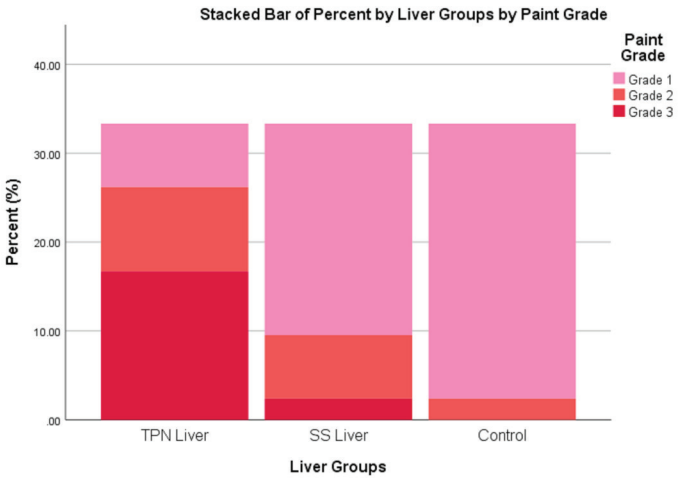
IHC staining of the skin and jejunum tissue revealed no statistically significant differences among the TPN, serum saline, and control groups (P > 0.05). Gonadal tissue: Table 2 summarizes the TERT staining grades. TERT level was significantly different among the groups (P < 0.05). Multiple comparisons revealed that TPN significantly increased TERT level in gonadal tissue sections (TPN, serum saline, control group grades of 0, 1, and 2, respectively, in 28.6%, 71.4%, and 71.4% of group 1 rabbits; 28.6%, 21.4%, and 21.4% of group 2 rabbits; and 42.9%, 7.1%, and 7.1% of group 3 rabbits) compared to other groups (Graph 1).

Table 2. The descriptive statistics of the gonadal tissue groups according to the TERT Level Paint Grade

	Groups			
TERT Level Paint Grade	TPN	Serum Saline	Control	P Value
Grade I	4 (28.6)[a]	10 (71.4)[b]	10 (71.4)[b]	0.048
Grade II	4 (28.6)[a]	3 (21.4)[b]	3 (21.4)[b]	
Grade III	6 (42.9)[a]	1 (7.1)[b]	1 (7.1)[b]	

a,b: Different superscript letters indicate a significant difference in each row (p<0.05); The data are given as n(%)

Liver tissue: Table 3 shows the TERT expression according to staining grade. TERT level was significantly different among the groups (P<0.05). Multiple comparisons revealed that TPN significantly increased TERT expression in the liver tissue (Grades 0, 1, 2: 21,4%, 71.4%, and 92.9%, respectively) compared with the serum saline (28.6%, 21.4%, and 7.1%, respectively) and control (50%, 7.1%, and 0%, respectively) groups (Graph 2).



Graph 1. TERT level in gonad tissue, expressed as percent immunohistochemical grade, according to the groups.

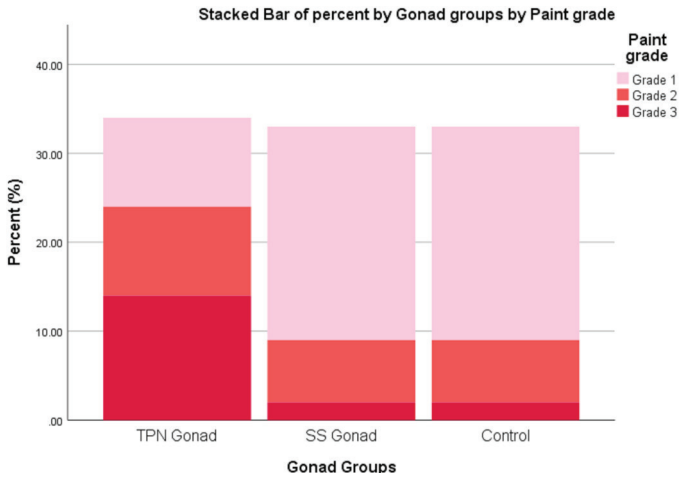
Serum telomerase level is shown in Table 4 according to group.

The multiple comparison tests revealed significant differences between the following group pairs: control (0.96c [0.86–1.13]) vs. TPN after (4.73d [0.96–11.49]); TPN before (0.98 c [0.86–1.60]) vs. TPN after (4.73d [0.96–11.49]); and serum saline before (1.05 [0.86–1.23]) vs. TPN after (4.73d [0.96–11.49]; P < 0.05; Graph 3)

Table 3. The descriptive statistics of the liver tissue groups according to the TERT Level Paint Grade

TERT Level	Liver tissue groups			P Value
Paint Grade	TPN	Serum Saline	Control	
Grade 1	3 (21.4%)[a]	10 (71.4%)[b]	13 (92.9%)[b]	
Grade 2	4 (28.6%)[a]	3 (21.4%)[a]	1 (7.1%)[a]	
Grade 3	7 (50%)[a]	1 (7.1%)[b]	0 (0%)[b]	0.001

a,b: Different superscript letters indicate a significant difference in each row (p<0.05); The data are given as n(%)

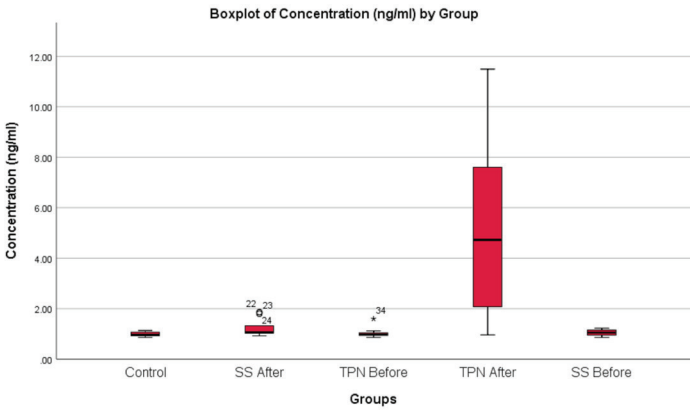


Graph 2. TERT level in the liver tissue, expressed as percent immunohistochemical grade, according to the groups.

Table 4. The descriptive statistics of the serum groups with respect to Telomerase Level Paint Grade. Kruskal- Wallis test

Variable	Control	SS After	TPN Before	TPN After	SS Before	p
Concentration (ng/ml) Median (Min-max)	0.96[c] (0.86-1.13)	1.06 (0.92-1.87)	0.98[c] (0.86-1.60)	4.73[d] (0.96-11.49)	1.05 (0.86-1.23)	<0.001

a: Different from SS After, b: Different from TPN Before, c: Different from TPN After, d: Different from SS Before



Graph 3. The distribution of the serum telomerase level paint grade percentages, according to the groups.

Although no significant difference was found between serum saline after (1.06 [0.92–1.87]) and TPN after (4.73d [0.96–11.49]), further analysis using the Mann–Whitney U-test revealed a significant between-group difference in concentration values (P < 0.001; Table 5).

No significant differences were found between the serum saline after group and the other groups.

Table 5. The descriptive statistics of the serum SS After with TPN After groups with respect to Telomerase Level. Mann-Whitney U test

Variable	SS After	TPN After	p
Concentration (ng/ml) (Median (Min-max)	1.06(0.92-1.87)	4.73[d] (0.96-11.49)	<0.001

DISCUSSION

Since 1969, when TPN usage became common in clinical practice, mortality has decreased in patients who cannot be fed enterally (22). The effects of TPN at the cellular level remain un-clear, including any effect of TPN on telomeres, which have several biological functions. Among these are the identification of DNA damage, protection of chromosomes against end-to-end fu-sion and recombination, facilitation of complete chromosomal proliferation, maintenance of func-tional chromosomal organization in the nucleus, regulation of gene expression, and level as a mo-lecular clock controlling cell growth and senescence (23).

Cong described the hTERT gene, which regulates telomerase level (23). Several studies of telomere kinetics have involved tumors (23–25). Cancer cells exhibit enhanced levels of telomer-ase level with increasing

numbers of chromosomes that carry single copies of the hTERT gene (25). Human papilloma virus initiates and enhances telomerase level in keratinocytes and mamma-ry epithelial cells (26). Steroid hormones increase telomerase level in normal human endometrium during the menstrual cycle and this level is correlated with endometrial cell proliferation (27). Tamoxifen, a nonsteroidal anti-estrogen drug, used to treat breast and colon cancers, reduces te-lomerase level (28). Androgens activate telomerase, but the underlying mechanism remains un-clear (29).

Bodnar et al have shown that telomerase level was regulated by reverse protein phosphory-lation of hTERT. Telomere level in peripheral blood mononuclear cells is increased by the addition of the protein kinase C (PKC) activator phorbol myristate acetate and inhibited by addi-tion of the PKC inhibitor bisindolylmaleimide I (30). Smoking reduces skin telomerase level and contributes to skin aging (31).

Blackburn et al found that serum telomerase level was an indicator of oxidative stress (32). For example, pesticides lead to the destruction of genetic material through excessive free radical production or altered antioxidant defense mechanisms (33). The effects of pesticides are associat-ed with an increase in serum telomerase level (34).

Several studies investigated the associations between TPN and apoptosis. Lipids content of TPN provide vital fatty acids. However, lipids induce hepatocyte apoptosis via a Bcl-2-mediated mitochondrial interaction (35). There are three principal apoptotic mechanisms: activation of death receptors, mitochondrial damage, and damage to nuclear chromosomes (36).

There is no study that examines the relationship between telomer kinetics and TPN. How-ever, there are studies examining the relationship between TPN and apoptosis. Yang et al. (37) investigated the association between apoptosis and TPN in mice. The height of the villus in the jejunum was used as the measure of apoptosis. The authors found a correlation between apop-tosis and TPN administration, which they attributed to a TPN-induced increase in interferon-ɤ (IFN-ɤ) expression, suggesting that the interferon plays a significant role in the Fas/Fas-ligand (Fas-L) interaction, leading to apoptosis. However, although oral intake was interrupted in the mice that received TPN, the study did not investigate the effect of starvation on apoptosis.

No previous studies have reported a correlation between osmolality and telomere level. Kuwahara et al. found that the administration of fluids with osmolality >600 mOsm/L caused endothelial cellular damage in the peripheral vein and that the phlebitis rate increased with the osmolality of the parenteral fluid (38). However, previous studies have shown that the negative effects of high-osmolality fluids were limited to the vein of administration (39).

The effect of a TPN solution on telomere level has not been studied previously. Since nu-clear and nucleolar TERT expression reflects significant telomerase level (32), the present study examined the effects of TPN on TERT expression in liver and gonadal tissues and serum telomer-ase level where telomere kinetics remain active. The intravenous administration of TPN for 10 days significantly enhanced TERT level in rabbit liver, testicular, and ovarian cells, thereby reduc-ing liver and gonadal cell senescence. Furthermore, we found that TERT increased serum te-lomerase level, revealing an increase in systemic DNA damage due to TPN toxicity. Although TPN has been shown to increase apoptosis by enhancing mitochondrial membrane permeability and the expressions of IFN-ɤ and Fas-L (37), we found that TPN exerted a positive effect on telomere kinetics in gonadal and liver cell nuclei and increased serum telomerase level. Cytoplas-mic TERT staining was also evident in all sections, consistent with the findings of Saretzki (40), who showed that mitochondria also exhibited telomerase level.

CONCLUSION

Our findings indicate that the TPN may have a positive impact on liver and gonadal telomere kinetics. However, we conclude that TPN increases the DNA damage all over the body. Future studies are planned to determine the TPN compounds underlying these effects.

Competing interests: The authors declare that they have no competing interest.

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