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Original Research

Serum levels of 8-hydroxy 2-deoxyguuanosine as a marker of DNA damage in healthy obese individuals

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Abstract

Background and Aims: Obesity is a serious and growing healthcare concern worldwide. It is associated with mortality and co-morbidity. We aimed to determine serum concentrations of 8-hydroxy-2`-deoxyguanosine and their association with body-mass index in healthy individuals. **Material and Method:** Fifty-nine healthy individuals were recruited from public places in this cross sectional study. Participants were divided into: obese group and non-obese group. Blood collected; serum and plasma were prepared. Glucose and glycated hemoglobin were assayed by standard methods using commercial kits. 8-hydroxy-2` -deoxyguanosine was determined by enzyme-linked immunosorbent assay. Data were analyzed using software Statistical Packages for the Social Sciences. **Results:** Data showed that median (interquartile levels) of serum 8-hydroxy-2`-deoxyguanosine was significantly higher in the obese subjects in comparison with controls. Moreover, a positive correlation was documented between level of serum 8-hydroxy-2`-deoxyguanosine and body-mass index in study participants. **Conclusions:** The study findings suggest that weight loss for obese individuals could reduce DNA damage and oxidative stress which underlie the pathogenesis of obesity-associated metabolic disorders.

Keywords: Healthy individuals, Obesity, Oxidative DNA damage, Oxidative stress, Reactive oxygen species.

Background and Aims

Obesity is a serious and growing healthcare concern worldwide [1, 2]. The prevalence of obesity was 55% in Europe, 46% in Eastern Mediterranean, 26.9% in Africa and 35.7% in USA [3, 4]. Obesity is associated with co-morbidities including: cardiovascular, diabetes mellitus, cancer, hypertension and metabolic disorders [5]. Moreover, Flegal KM et al., [6] reported that high level of obesity was associated with increased relative risks of mortality, which is partially attributed to increased risk for chronic diseases especially when obesity starts at an early age [7]. One consequence of obesity is the insulin metabolic dysregulation for free fatty acids and glucose [8].

Cellular respiration and multiple enzyme systems such as P450 monoxygenase system can generate reactive radicals [9]. Although the human body has defense mechanisms against these reactive radicals, sometimes they may not function optimally and result in oxidative damage, as is seen in obesity and its associated complications [10–12]. In obese subjects, oxygen-derived radicals—such as reactive oxygen species (ROS)—and decreased antioxidants disrupt the redox system [13, 14]. ROS, oxygen-containing molecules with or without unpaired electrons, are highly reactive in tissues [15]. They oxidize macromolecules like lipids, proteins and DNA [11]. Thus, oxidative damage markers can be identified: malondialdehyde for lipid peroxidation; advanced glycosylated end

products for protein oxidative modification; and 8-hydroxy-2'-deoxyguoanosine (8-OHdG) for oxidatively modified products of DNA [16]. Different forms of DNA damage in human include: single strand breaks, double strand breaks, mis-pairing, and base modification [17]. An example of the latter is 8-OHdG, which is an oxidized purine base in DNA [18]. It is a sensitive biomarker for oxidative DNA damage [19]. Researchers documented increased concentration of 8-OHdG in different human specimens in many diseases including diabetes, cardiovascular, cancer etc [20].

Despite the huge literature of studying 8-OHdG in obesity associated co-morbidities, limited number of studies explored 8-OHdG in healthy obese subjects. Herein, we hypothesized that obese healthy individuals have increased serum 8-OHdG than non-obese subjects. Therefore, the study aims to determine serum 8-OHdG in healthy obese individuals in comparison with non-obese healthy individuals. We will also examine the relationship between 8-OHdG and body mass index (BMI) among healthy obese individuals.

Materials and Methods

Study Design and participants

In this cross sectional study, fifty-nine healthy individuals were recruited from public places. Based on World Health Organization criteria of obesity [21], participants were further divided into two group: obese group and non-obese group. The study excluded participants who reported they had chronic diseases such as: diabetes mellitus, hypertension, or cardiovascular disease. In addition, random blood glucose levels <11.1 mmol/l and glycated hemoglobin (HbA1c) <5.5% were considered as confirmatory criteria to enroll participants in the study.

Laboratory, anthropometric and clinical data collection

Body weight and height were measured for each participant. BMI was calculated as body weight (kilogram) divided by height (meter) squared. Participants with BMI ≥30 Kg/m^2 were classified as obese while those with BMI <30 Kg/m^2 were considered as non-obese.

After verbal consent, each participant agreed to give blood samples in plain and heparinized vacutainers. Blood samples in vacutainers was allowed to clot, then centrifuged at 3000 rpm for 15 minutes to get serum. While blood in heparinized vacutainers was spun at 3000 rpm for 10 minutes to get plasma.

We used commercial kit manufactured by Human Diagnostics, Wiesbaden, Germany for measurement of blood glucose. The principle is based on Glucose oxidase-glucose peroxidase method. Kits based on immunoturbidmetric method were used to assay HbA1c. They were purchased from Vital Diagnostic, Italy. The serum levels of 8-OHdG were determined by using a competitive enzyme-linked immunosorbent assay kit which was obtained from the Northwest Life Science Specialties, U.S.A

Statistical Analysis

IBM SPSS statistics (version 23) software was used to analyze data. Variables were compared with the normal distribution using the skewness/kurtosis measures. Nonparametric methods were applied when data were not normally distributed. Descriptive statistics (mean ± standard deviation or number (percentage) were used for continuous or categorical variable respectively. In addition, level of 8-OHdG was expressed as median (interquartile) and mean ± standard deviation.

Differences between the two groups (obese vs. non-obese) were compared using chi square, unpaired t-test or the Mann-Whitney U-test where appropriate. Spearman correlation test was performed to examine the relationship BMI and 8-OHdG. We considered results to be significant at $p<0.05$.

Ethical Consideration

The study was conducted in accordance with the Declaration of Helsinki. After thorough explanation of the goals of the study, each

participant gave verbal consent. Participation was voluntary and confidentiality of all participants was maintained as no names were requested.

Results

Characteristics & Laboratory investigations of study participants

Data show no significant difference in the gender, age, education, and smoking status ($p>0.05$) between the two groups. Although not significant, apparently male represented the majority of non-obese group while female did in obese group. All participants in both groups had blood glucose level and HbA1c within normal range (Table 1).

Measurement of 8-OHdG in study participants

Table 2 shows that the median (interquartile levels) of 8-OHdG was significantly higher in the obese group compared to controls ($p<0.05$).

Relation between BMI and 8-OHdG in study participants

Spearman correlation analysis shows that BMI and 8-OHdG were significantly and positively correlated ($r= 0.266$, $p=0.045$) (Fig 1).

Discussion

Normal cellular metabolism as well as pathophysiological process could result in production of oxidant species [22]. Oxidative damage

Table 1: Summarizes data for characteristics of the study participants.

Variable	Obese (n=23)	Non-obese (n=36)	p-value
Male, n (%)	9 (39.1)	21 (58.3)	0.150
Age, years	46.8±9.1	42.8±11.6	0.159
Weight, Kg	87.0±14.9	67.2±9.3	0.000*
Height, cm	153.4±20.5	163.8±7.1	0.007*
BMI, kg/m^2	35.7±6.1	25.7±2.4	0.000*
Education, University or high Illiterate	 6 (26.1) 8 (34.8)	 16 (44.4) 10 (27.8)	 0.276
Smoking, n (%)	5 (21.7)	5 (13.9)	0.433
Blood glucose, mmol/l	5.22±0.40	5.74±0.34	0.349
Hb A1c, %	4.7±0.30	5.2±0.24	0.201

Data are represented as mean± SD or number (%)
BMI: Body mass index; HbA1c: glycosylated hemoglobin

Table 2: Comparison of serum 8-OHdG levels between obese and non-obese groups.

	Obese		Non-obese	
	Means ± SD	Median (IQR)	Means ± SD	Median (IQR)
8OHdG (ng/ml)	0.638±0.10*	0.51 (0.32–0.71)**	0.405±0.05	0.325 (0.19–0.49)

Data presented as mean ± SD, median (IQR).
8-OHdG: 8-hydroxy-2`-deoxyguanosine; IQR: interquartile range.
* $p=0.025^{*}$, ** $p= 0.023$

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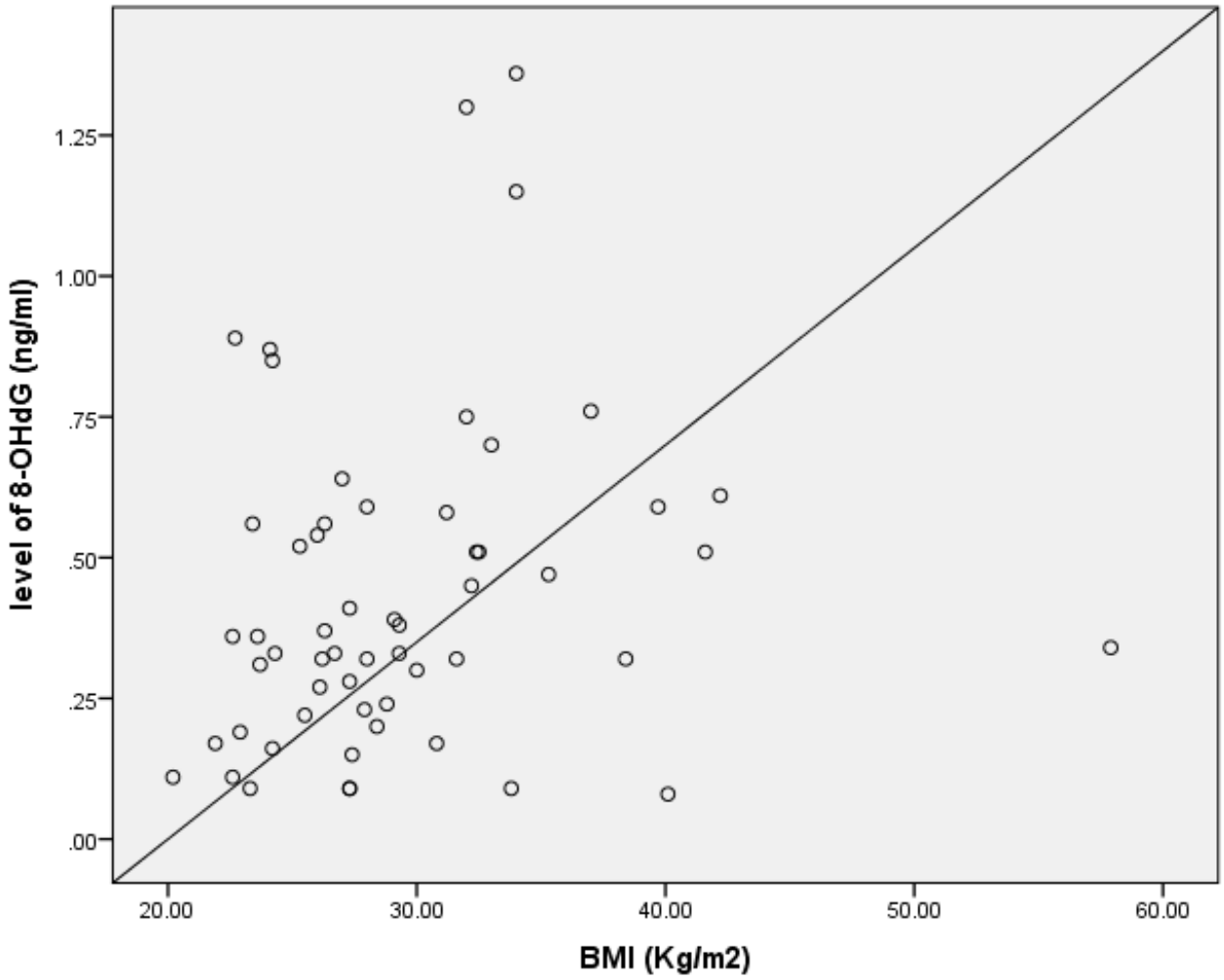

Figure 1: Positive correlation between level of serum 8-OHdG (ng/ml) and BMI (Kg/m^2) in all participants (r = 0.266*, p = 0.045)

to DNA is the most critical modification compared to other cellular biomolecules notably: proteins and lipids [23]. 8-OHdG is a DNA guanine residue modification produced by ROS [24].

We have shown here that the median (interquartile levels) of serum 8-OHdG was significantly higher in the obese subjects in comparison with controls ($p<0.05$). Moreover, data from present study show a statistically significant positive correlation between level of serum 8-OHdG (ng/ml) and BMI (Kg/m^2) in study participants.

Obesity is a multifactorial complex progressive disorder that characterized by excessive fat accumulation as a result of surplus caloric intake or sedentary lifestyle [25]. In obese individuals, the adipose tissue is a source for ROS that released into peripheral blood [26]. The increased release of free fatty acids from over-accumulated fat leads to activation of NADPH oxidase in adipocytes and ultimately in ROS production. NAD phosphate (NADPH) oxidase is an enzyme involved in nutrient-based ROS generation [27]. Direct reaction of ROS with DNA produces a multiplicity of modifications of base and sugar in DNA which if left unrepaired, can lead to diseases [28, 29]. Also, the DNA damage could induce inflammation in visceral adipose tissue which result in development of systemic insulin resistance and lipids dysregulation that accelerate various disease states in individuals with obesity [30]. In support for this approach, Dandona P et al., reported significant reduction in ROS and oxidative stress occurred in nondiabetic obese subjects after short period of dietary restriction and weight loss [31].

In agreement with our results, a study reported a significant increase in serum 8-OHdG in obese subjects (848.5G103 pg/ml;

p<0.001) compared with the lean subjects (196.5G327 pg/ml) [32]. Another study reported that patients with obesity present increased concentrations of oxidative stress by-products such 8-oxo-7,8-dihydro-2'-deoxyguanosine, which is DNA oxidized and highly mutagenic base [33]. In support to these findings, two studies reported high level of mean DNA damage (measured by the comet assay) in obese women compared with non-obese women [34, 35].

Interestingly, Kocael A et al [36] reported significantly (p<0.001) decreased levels of serum 8-OHdG in morbidly obese adults (BMI>40 Kg/m^2) after six months of bariatric surgery which concurrent with efficient weight loss. Authors concluded that the systemic oxidative DNA damage was increased by the morbid obesity in spite that pre- and postoperative serum levels did not correlate with their corresponding weight and BMI. Another study reported increased serum 8-oxo-deoxyguanosine in morbid obese patients when compared to controls and after one year of bariatric surgery [37].

In contrary to our findings, Donmez-Altuntas H et al., [38] reported that plasma concentrations of 8-OHdG in obese subjects were lower compared with normal weight controls (p<0.05). In addition, authors did not observe significant correlation between plasma concentrations of 8-OHdG and BMI (p>0.05). They attributed their findings to efficient repair of the 8-OHdG lesions by base-excision repair pathway. Also, Sakano et al., [39] reported that urinary 8-OHdG was negatively associated with BMI in healthy Japanese subjects.

Conclusions

We have shown here that the serum level of an oxidative DNA damage marker, 8-OHdG, was increased in healthy obese individuals compared to non-obese. There was a significant positive association between DNA damage and BMI. Since weight loss is a manageable and modifiable factor, the study findings suggest that weight loss for obese individuals could reduce DNA damage and oxidative stress which underlie the pathogenesis of obesity-associated metabolic disorders.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Flier JS. Obesity wars: molecular progress confronts an expanding epidemic. *Cell*. 116(2):337–50, 2004.
2. Andreassi MG, Barale R, Iozzo P, Picano E. The association of micronucleus frequency with obesity, diabetes and cardiovascular disease. *Mutagenesis*. 26(1):77–83, 2011. doi: 10.1093/mutage/geq077
3. Ahmed MH, Ali YA, Awadalla H, Elmadhoun WM, Noor SK, Almobarak AO. Prevalence and trends of obesity among adult Sudanese individuals: Population based study. *Diabetes Metab Syndr*. 11(2):S963–S967, 2017. doi: 10.1016/j.dsx.2017.07.023
4. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity among adults: United States, 2011-2012. *NCHS Data Brief*. 131:1–8, 2013.
5. Lee JY, Lee YR, Kim HR, Myong JP, Kang MY. Trends in Obesity Prevalence by Occupation Based on Korean National Health and Nutrition Examination Survey From 1998 to 2015. *Saf Health Work*. 11(1):97–102, 2020. doi: 10.1016/j.shaw.2019.08.003
6. Flegal KM, Graubard BI, Williamson DF, Gail MH. Excess deaths associated with underweight, overweight, and obesity. JAMA. 293(15):1861–7, 2005.
7. Musaad S, Haynes EN. Biomarkers of obesity and subsequent cardiovascular events. *Epidemiol Rev*. 29:98–114, 2007.
8. Andreassi MG, Barale R, Iozzo P, Picano E. The association of micronucleus frequency with obesity, diabetes and cardiovascular disease. *Mutagenesis*. 26(1):77–83, 2011. doi: 10.1093/mutage/geq077
9. Auten RL, Davis JM. Oxygen toxicity and reactive oxygen species: the devil is in the details. *Pediatr Res*. 66(2):121–7, 2009. doi: 10.1203/PDR.0b013e3181a9eafb
10. De Bont R, van Larebeke N. Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis. 19(3):169–85, 2004.
11. Suzuki K, Ito Y, Ochiai J, Kusuhara Y, Hashimoto S, Tokudome S, Kojima M, Wakai K, Toyoshima H, Tamakoshi K, Watanabe Y, Hayakawa N, Maruta M, Watanabe M, Kato K, Ohta Y, Tamakoshi A. JACC Study Group. Relationship between obesity and serum markers of oxidative stress and inflammation in Japanese. *Asian Pac J Cancer Prev*. 4(3):259–66, 2003.
12. Schulze PC, Lee RT. Oxidative stress and atherosclerosis. *Curr Atheroscler Rep*. 7(3):242–8, 2005.
13. Usman M, Volpi EV. DNA damage in obesity: Initiator, promoter and predictor of cancer. *Mutat Res*. 778:23–37, 2018. doi: 10.1016/j.mrrev.2018.08.002
14. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *Int J Obes (Lond)*. 30(3):400–18, 2006.
15. Vincent HK, Innes KE, Vincent KR. Oxidative stress and potential interventions to reduce oxidativestress in overweight and obesity. *Diabetes Obes Metab*. 9(6):813–39. 2007.
16. Makromolekula N. New Markers of Oxidative Damage to Macromolecules. JMB. 27: 1–16, 2008. doi: 10.2478/v10011-007-0049-x

 https://doi.org/10.46389/rjd-2020-1053

17. Gray K, Bennett M. Role of DNA damage in atherosclerosis–bystander or participant? Biochem Pharmacol. 82(7):693–700, 2011. doi: 10.1016/j.bcp.2011.06.025.
18. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *Int J Obes (Lond)*. 30(3):400–18, 2006.
19. Al-Aubaidy HA, Jelinek HF. Oxidative DNA damage and obesity in type 2 diabetes mellitus. *Eur J Endocrinol*. 164(6):899–904, 2011. doi: 10.1530/EJE-11-0053
20. Koide S, Kinoshita Y, Ito N, Kimura J, Yokoyama K, Karube I. Determination of human serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) by HPLC-ECD combined with solid phase extraction (SPE). *J Chromatogr B Analyt Technol Biomed Life Sci*. 878(23):2163–7, 2010. doi: 10.1016/j.jchromb.2010.06.015
21. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser*. 894:i–xii, 1–253, 2000.
22. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol Rev*. 94(2):329–54, 2014. doi: 10.1152/physrev.00040.2012
23. Cooke MS, Evans MD, Dove R, Rozalski R, Gackowski D, Siomek A, Lunec J, Olinski R. DNA repair is responsible for the presence of oxidatively damaged DNA lesions in urine. *Mutat Res*. 574(1–2):58–66, 2005.
24. Shin CS, Moon BS, Park KS, Kim SY, Park SJ, Chung MH, Lee HK. Serum 8-hydroxy guanine levels are increased in diabetic patients. *Diabetes Care*. 24(4):733–7, 2001.
25. Nijhawan P, Arora S, Behl T. Intricate role of oxidative stress in the progression of obesity. *Obesity Medicine*. 15: 100125, 2019.
26. Marseglia L, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rosa G, Gitto E, Arrigo T. Oxidative stress in obesity: a critical component in human diseases. *Int J Mol Sci*. 16(1):378–400, 2014. doi: 10.3390/ijms16010378
27. Matsuda M, Shimomura I. Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes,hypertension, dyslipidemia, atherosclerosis, and cancer. *Obes Res Clin Pract*. 7(5):e330–41, 2013.
28. Andreassi MG. DNA damage, vascular senescence and atherosclerosis. *J Mol Med (Berl)*. 86(9):1033–43, 2008. doi: 10.1007/s00109-008-0358-7
29. Noren Hooten N, Ejiogu N, Zonderman AB, Evans MK. Association of oxidative DNA damage and C-reactive protein in women at risk for cardiovasculardisease. *Arter ioscler Thromb Vasc Biol*. 32(11):2776–84, 2012. doi: 10.1161/ATVBAHA.112.300276
30. Shimizu I, Yoshida Y, Suda M, Minamino T. DNA damage response and metabolic disease. *Cell Metab*. 20(6):967–77, 2014. doi: 10.1016/j.cmet.2014.10.008
31. Dandona P, Mohanty P, Ghanim H, Aljada A, Browne R, Hamouda W, Prabhala A, Afzal A, Garg R. The suppressive effect of dietary restriction and weight loss in the obese on the generation of reactive oxygen species by leukocytes, lipid peroxidation, and protein carbonylation. *J Clin Endocrinol Metab*. 86(1):355–62, 2001.
32. Al-Aubaidy HA, Jelinek HF. Oxidative DNA damage and obesity in type 2 diabetes mellitus. *Eur J Endocrinol*. 164(6):899–904, 2011. doi: 10.1530/EJE-11-0053
33. Cerda C, Sanchez C, Climent B, Vazquez, A, Iradi A, El Amrani F; Bediaga A, Saez GT. Oxidative stress and DNA damage in obesity-related tumorigenesis. *Adv. Exp. Med. Biol*. 824: 5–17, 2014.
34. Zaki M, Basha W, El-Bassyouni HT, El-Toukhy S, Hussein T. Evaluation of DNA damage profile in obese women and its association to risk of metabolic syndrome, polycystic ovary syndrome and recurrent preeclampsia. *Genes Dis*. 5(4):367–373, 2018. doi: 10.1016/j.gendis.2018.03.001. eCollection 2018 Dec
35. Włodarczyk M, Jabłonowska-Lietz B, Olejarz W, Nowicka G. Anthropometric and Dietary Factors as Predictors of DNA Damage in Obese Women. *Nutrients*. 10(5). pii: E578, 2018. doi: 10.3390/nu10050578
36. Kocael A, Erman H, Zengin K, Kocael PC, Korkmaz GG, Gelisgen R, Taskin M, Ersan Y, Uzun H. The effects on oxidative DNA damage of laparoscopic gastric band applications in morbidly obese patients. *Can J Surg*. 57(3):183–7, 2014.
37. Monzo-Beltran L, Vazquez-Tarragón A, Cerdà C, Garcia-Perez P, Iradi A, Sánchez C, Climent B, et al. One-year follow up of clinical, metabolic and oxidative stress profile of morbid obese patients after laparoscopic sleeve gastrectomy. 8-oxo-dG as a clinical marker. *Redox Biol*. 12:389–402, 2017. doi: 10.1016/j.redox.2017.02.003
38. Donmez-Altuntas H, Sahin F, Bayram F, Bitgen N, Mert M, Guclu K, Hamurcu Z, Arıbas S, Gundogan K, Diri H. Evaluation of chromosomal damage, cytostasis, cytotoxicity, oxidative DNA damage and their association with body-mass index in obese subjects. *Mutat Res Genet Toxicol Environ Mutagen*. 771:30–6, 2014. doi: 10.1016/j.mrgentox.2014.06.006. Epub 2014 Jun 28.
39. Sakano N, Takahashi N, Wang DH, Sauriasari R, Takemoto K, Kanbara S, Sato Y, Takigawa T, Takaki J, Ogino K. Plasma 3-nitrotyrosine, urinary 8-isoprostane and 8-OHdG among healthy Japanese people. *Free Radic Res*. 43(2):183–92, 2009. doi: 10.1080/10715760802663124