ORIGINAL ARTICLE

DNA Damage and Obesity Among Faculty of Pharmacy Students

Farah Natasha Zalizan, Mizaton Hazizul Hasan, Maziana Mahamood

Faculty of Pharmacy, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, Bandar Puncak Alam, 42300 Selangor, Malaysia.

ABSTRACT

Introduction: Obesity is a major concern on a global scale, particularly in Malaysia. It causes a physiological imbalance in the regulation and normal functioning of adipose tissue, leading to other medical issues such as hyperglycaemia, dyslipidaemia, and inflammation. These conditions trigger the production of oxidative stress, which is worsened by a decrease in antioxidant defence systems in obese patients. **Methods:** This study was conducted to evaluate body mass index (BMI), waist-to-hip ratio (WHR), the level of subcutaneous fat in the whole body, trunk, leg, and arm and visceral fat of the subjects, and their relationship with DNA damage parameters among the students of Faculty of Pharmacy (n=89) in Universiti Teknologi MARA (UiTM). DNA damage was assessed using Comet Assay. **Results:** No significant differences ($p>0.05$) in the tail length, tail moment, olive moment, and the percentage of DNA in tail were observed between groups, although greater value of DNA damage parameters (mean ± SD) was seen in overweight group. There was also no correlation between the anthropometric measurements and DNA damage ($p>0.05$). In summary, it can be concluded that there was no significant difference in the levels of DNA damage among the normal and overweight/obese group with no correlation between DNA damage and BMI ($p>0.05$). **Conclusion:** Further study should be conducted to understand the mechanism/s that contributed to this condition.
Malaysian Journal of Medicine and Health Sciences (2023) 19(SUPP18)52-59. doi:10.47836/mjmhs19.s18.8

Keywords: Obesity, Body mass index (BMI), Waist-to-hip ratio (WHR), Body fat, DNA damage

Corresponding Author:
Maziana Mahamood, PhD
Email: maziana2795@uitm.edu.my
Tel: +603 32584787

INTRODUCTION

The worldwide prevalence of obesity has risen dramatically. Malaysia has the highest rate of obesity and overweight among Asian countries with half of the adult population either being overweight or obese. According to the National Health and Morbidity Survey (NHMS) 2019 (1), out of 50.1% of Malaysian adults, 30.4% were overweight and 19.7% obese. There is a wide spectrum of medical complications and one of the consequences is the increase of noncommunicable diseases such as cardiovascular disease (2). Teenage obesity is associated with a higher chance of obesity, premature death, and disability in adulthood. But in addition to increased future risks, obese adolescents experience breathing difficulties, increased risk of fractures, hypertension, early markers of cardiovascular disease (3), insulin resistance and psychological effects (4).

Obesity is intricately linked to imbalances in reactive oxygen species (ROS)-mediated signalling and antioxidative defence, causing oxidatively damaged DNA. If this is the case, our hypothesis is that the increase in body mass index (BMI) is an important contributor to DNA damage, including in teenagers that also includes university students. It is estimated that there are 1.2 billion adolescents in the global population. The Department of Statistics Malaysia estimated the population aged 15-64 years old which includes the adolescent group, increased from 69.7 per cent in 2018 to 70.0 per cent in 2019. Globally, there are 1.2 billion adolescents aged 15–24 years representing more than 16% of the world's population and the numbers increase (5). Therefore, it is significant to use a proactive approach and emphasizes the importance of early prevention

of disease. That would translate into monetary saving as less ringgit would be spent on the management of chronic illnesses that would otherwise be experienced by them. Increased oxidative stress, DNA damage and obesity prevalence are amongst the underlying causes of chronic diseases (3). This warranted the need for studies to determine whether improvements in DNA damage and health condition can be rescued by diet and weight management plans.

Weight reduction to a certain extent can decrease oxidative stress and DNA damage in obese people (6). Weight loss intervention is one of the non-pharmacological treatments that has been studied in reducing oxidative DNA damage in obese individuals. There are two common ways of reducing extra body weight. An energy restriction diet is the key factor of weight loss (7), followed by macronutrient composition, based on a meta-analysis of numerous diet programmes (8). A link between oxidative stress and obesity has been described frequently in the literature (9). Some studies showed the relation between obesity with DNA damage in diseases like diabetes type 2, polycystic ovary syndrome (10) or metabolic syndrome (11), which are usually associated with elevated body mass index (BMI). A study by Bankoglu et al. (12) showed a significant correlation between weight reduction in obese patients after bariatric surgery with DNA damage levels. Therefore, the present research is proposed to address gaps in knowledge of oxidative DNA damage and overweight/ obese students of the Faculty of Pharmacy, UiTM. The objectives of this study were to measure (i) the overweight/ obesity prevalence among students of Faculty of Pharmacy, UiTM (ii) the oxidative DNA damage in students of Faculty of Pharmacy, UiTM (iii) the association between overweight/ obesity and DNA damage. The subjects were chosen among the students of Faculty of Pharmacy (UiTM) due to the potential roles for pharmacy students in combatting this health issue and the involvement in the health care system.

MATERIALS AND METHODS

Subjects

The study included 89 subjects (66 females and 23 males). All of them are categorised based on their BMI which includes obese (>30 kg/m^2), overweight (25-29.9 kg/m^2), normal weight (18.5-24.9 kg/m^2) and underweight (<18.5 kg/m^2). All subjects were second-year and third-year pharmacy students from the Faculty of Pharmacy in UiTM Puncak Alam. The participants were fully informed about the study and were asked to sign an informed consent form on the day of the research conducted. The study was approved by the Research Ethics Committee of Universiti Teknologi MARA [REC/12/2021 MR/10680]. Besides, during the sampling day, the subjects were provided with a set of questionnaires regarding the subject information. Inclusionary criteria for this study included students of the Faculty of Pharmacy, UiTM aged between 19 to 25 years, generally healthy. Exclusion criteria include those who were incapable of self-care or those with debilitating conditions (cardiovascular conditions, impaired hearing or sight, difficulties with mobility).

Anthropometric measurements

The anthropometric measurements include height (cm), weight (kg), BMI (kg/m^2), subcutaneous fat of the whole body, trunk, leg and arms, visceral fat and waist-to-hip ratio (WHR) measured. All variables except height were measured by using a body composition analyser (Omron, Japan). While height, it was measured by a height-measuring scale or portable stadiometer (Seca, Germany). The subjects were instructed to take off their shoes and any other clothing that might add weight and height before being weighed and measuring height. The height data was included in the Omron body composition analyser to get the BMI value. A BMI less than 18.50 kg/m^2 was considered underweight, a BMI between 18.50 and 24.90 kg/m^2 was considered normal, a BMI between 25.00 and 29.90 kg/m^2 was considered overweight, and a BMI of 30.00 kg/m^2 or higher was considered obese. Obesity is further grouped as class I if the BMI is between 30.00 and 34.90 kg/m^2, class II if it is between 35.00 and 39.99 kg/m^2, and class III if it is greater than 40.00 kg/m^2. Next, WHR was measured while the subject was standing with the hand fully extended to the side and breathing normally. The waist circumference (cm) was measured using a measuring tape from the point halfway between the costal margin and iliac crest in the mid-axillary line. Hip circumference (cm) was measured at the widest point of hip by using the same measuring tape. Then, WHR value was calculated by dividing waist circumference with hip circumference (13). Those with a WHR greater than 0.90 for men and greater than 0.85 for women were assumed abdominally obese.

Blood sampling (Finger-prick)

Blood sampling was collected at the same time as the clinical evaluation (height and weight). Blood samples from the subjects were collected by using a finger prick. The least calloused fingertip was chosen, and the fingertip was cleaned with an alcohol swab before performing a finger prick. A new sterile lancet was used for each subject. It was conducted by placing the lancet off centre and were pressed firmly against the finger to puncture the skin.

Comet assay

The comet assay or alkaline single cell gel electrophoresis was carried out to assess DNA damage. The cells were embedded in a thin agarose gel on a microscope slide and were then lysed and electrophoresed under low voltage (14). The electric current pulled the charged DNA from the nucleus such that relaxed and broken DNA fragments migrated further (15). The DNA was then stained with a fluorescent dye (ethidium bromide) and resembled a comet with a head and tail which

was observed by a fluorescence microscope (16). The intensity of the comet tail reflects the number of DNA breaks (17).

DNA damage was measured in leukocytes by a modified method of Singh et al. (18). Blood sample (15 µl) from the fingertip was put inside the Eppendorf tube containing 0.6% Low Melting Point Agarose (LMPA). It is generally used when the isolation of separated DNA fragments is desired. Then, the mixture of blood and LMPA (75 µl) were transferred quickly onto the slide that has been coated with 1.5% of Normal Melting Point Agarose (NMPA) and was covered with a cover slip. Next, the half-frosted slide was dipped into the NMPA and was left until solidified. The coated slide containing the mixture of blood and LMPA was placed on the tray filled with ice to solidify the gel before being immersed into the lysing solution. The slides were placed on the electrophoresis plane side by side and were immersed in fresh alkaline electrophoresis buffer which consisted of 300 mM NaOH and 1 mM Na2EDTA (pH 13) for 20 minutes to allow the unwinding of the DNA. An electric current of 25 volts and 300 mA was applied for 20 minutes to electrophoresis the DNA. The slides were then neutralised three times with 0.4 M Tris buffer (pH 7.5) and were stained with ethidium bromide (20 µg/mL) before covering them with the coverslips. Finally, the slides were analysed using a fluorescence microscope 1 or two days after the experiment. DNA damage was assessed by cell scoring using a microscope (Carl Zeiss, Germany) with an automated scanning platform and analysis system, Metafer 4 software programs.

Statistical Analysis

All data was expressed as mean ± Standard Deviation (SD). All statistical evaluations were performed using SPSS (Windows version 22.0, SPSS, Inc., Chicago, IL). The significance was analysed by Kruskal-Wallis test and a probability of at least $p<0.05$ was considered statistically significant. The correlation between the anthropometrics data and DNA damage (tail length) was measured using Spearman's correlation test.

RESULT

Demographic Data

The demographic characteristics of the subjects were shown in Table I. The subjects who participated in the study were 89 students consisting of both gender; male (n=23) and female (n=66); aged between 20 to 24 years old.

Anthropometric parameters

Table I : Demographic characteristics.

Demographic characteristics	N	(%)
Male		
Age (years)		
21	11	12.36
22	8	8.99
23	1	1.12
24	3	3.37
Female		
Age (years)		
20	1	1.12
21	15	16.85
22	23	25.85
23	8	8.99
24	19	21.35
TOTAL	**89**	100

Table II shows the anthropometric measurements such as BMI (kg/m^2), WHR, subcutaneous fat for whole body, trunk, leg and arm and visceral fat in mean ± SD. Demographic characteristics like age are also presented in the table in mean ± SD. All subjects (66 females and 23 males; mean age ± SD, 22.26 ± 1.15) are categorised based on their BMI which include 1 obese subject (1 male, age 21), 17 overweight subjects (14 females and 3 males; mean age ± SD, 22.24 ± 1.15), 52 normal weight subjects (36 females and 16 males; mean age ± SD, 22.29 ± 1.19) and 19 underweight subjects (16 females and 3 males; mean age ± SD, 22.26 ± 1.10).

Table II: Anthropometric measurements and age in the study population, grouped according to weight status (mean ± SD)

	Under-weight (<18.4 kg/m^2)	Normal (18.5-24.9 kg/m^2)	Over-weight (25.0-29.9 kg/m^2)	Obese (>30.0 kg/m^2)
TOTAL				
Subjects (N)	19	52	17	1
BMI (kg/m^2)	16.95 ± 1.17	21.40 ± 1.98	26.24 ± 1.2	34.2
Age (years)	22.26 ± 1.10	22.29 ± 1.19	22.24 ± 1.15	21
Waist/Hip (WHR)	0.74 ± 0.04	0.77 ± 0.51	0.80 ± 0.05	0.97
Whole body fat	17.61 ± 3.89	20.84 ± 5.84	28.78 ± 4.95	24.4
Trunk fat	14.43 ± 3.68	17.76 ± 5.08	25.08 ± 4.32	22.9
Leg fat	28.27 ± 6.08	31.48 ± 9.02	42.00 ± 7.82	35

CONTINUE

Table II: Anthropometric measurements and age in the study population, grouped according to weight status (mean ± SD) (cont.)

	Under-weight (<18.4 kg/m²)	Normal (18.5-24.9 kg/m²)	Over-weight (25.0-29.9 kg/m²)	Obese (>30.0 kg/m²)
TOTAL				
Arm fat	33.32 ± 8.09	35.04 ± 10.79	44.64± 8.77	34.5
Visceral fat	1.05 ± 0.23	3.63 ± 1.75	6.88 ± 1.73	18
Female				
Subjects (N)	16	36	14	
BMI (kg/m²)	16.86 ± 1.26	21.08 ± 2.00	26.19 ± 1.37	
Age (years)	22.19 ± 1.11	22.56 ± 1.16	22.14 ± 1.17	
Waist/Hip (WHR)	0.72 ± 0.03	0.75 ± 0.04	0.79 ± 0.04	
Whole body fat	18.83 ± 2.32	24.10 ± 2.83	30.46 ± 2.10	
Trunk fat	15.40 ± 2.81	20.44 ± 3.06	26.50 ± 2.38	
Leg fat	30.40 ± 3.00	36.74 ± 3.94	44.69 ± 3.88	
Arm fat	36.26 ± 4.09	41.49 ± 4.32	47.64 ± 4.31	
Visceral fat	1.0 ± 0.0	2.86 ± 1.22	6.50 ± 1.35	
Male				
Subjects (N)	3	16	3	1
BMI (kg/m²)	17.40 ± 0.44	22.13 ± 1.78	26.47 ± 0.25	34.20
Age (years)	22.67 ± 1.16	21.69 ± 1.08	22.67 ± 1.16	21
Waist/Hip (WHR)	0.81 ± 0.06	0.82 ± 0.04	0.86 ± 0.06	0.97
Whole body fat	11.03 ± 4.35	13.52 ± 3.85	20.97 ± 7.49	24.4
Trunk fat	9.27 ± 3.91	11.71 ± 3.09	18.47 ± 5.76	22.9
Leg fat	16.86 ± 5.85	19.63 ± 4.95	29.43 ± 10.21	35
Arm fat	17.67 ± 5.15	20.50 ± 5.21	30.63 ± 11.76	34.5
Visceral fat	1.33 ± 0.56	5.31 ± 1.58	8.67 ± 2.52	18

In overweight and obese subjects, the WHR, subcutaneous fat (whole body, trunk, leg, arm) and visceral fat showed great value compared to underweight and normal weight subjects. Obesity is referred to by a variety of terms, including abdominal obesity, abdominal adiposity, body fat percentage, and obesity predictors (36). Body mass index (BMI) is the widely used parameter to measure abdominal obesity (36). However, the WHR was suggested as another measure of abdominal obesity. Thus, an increase in BMI lead to increase in WHR. Both subcutaneous fat and visceral fat also increase with the increase of BMI because the percentage of adipose tissue is higher in women, the elderly and overweight individuals (37). From the data collected, female showed high value in adipose tissue compared to male.

Comet Assay

In this study, DNA damage parameters such as tail length, tail moment, olive moment and % of DNA in tail in blood sample of obese, overweight, normal weight and underweight subjects were evaluated. DNA damage parameters were measured by using comet assay. These parameters are directly proportional to the DNA damage. Figures 1 and 2 below show the image of the comet assay.

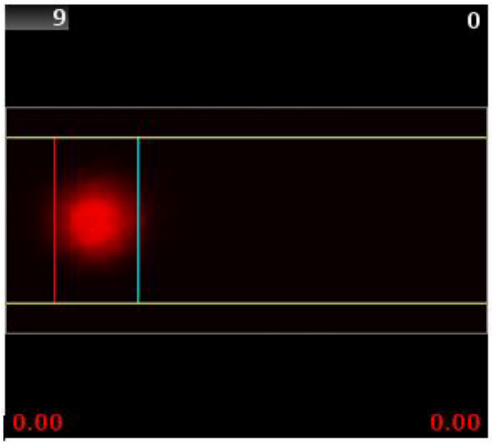

Figure 1: Normal cell

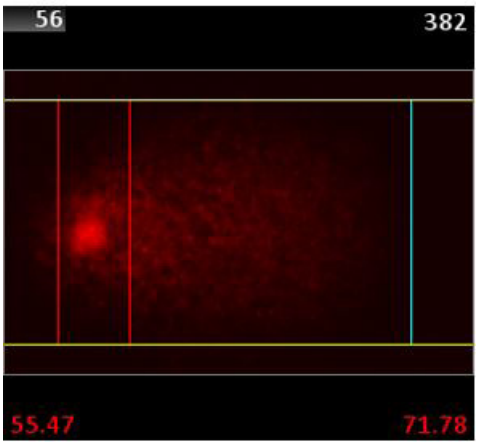

Figure 2: Damaged cell

Based on Shapiro-Wilk test, the distribution of the data for DNA damage parameters (tail length, tail moment, olive moment and % of DNA in tail) significantly not normally distributed ($p<0.05$). Thus, non-parametric statistical test (Kruskal-Wallis test and Spearman correlation test) was used in the analysis. Table III shows statistical comparisons of tail length, tail moment, olive moment and % of DNA in tail with BMI status.

Tail length, tail moment, olive moment and % of DNA in tail were found no statistically significant difference

between the four independent groups in the continuous outcome variable. According to gender, both in male and female also showed no statistically significant difference between the four independent groups in the continuous outcome variable damage ($p<0.05$, Kruskal-Wallis test).

Table III: Statistical comparisons among underweight, normal weight, overweight and obese subjects for DNA damage parameters (mean ± SD)

Parameter	Weight Status				*p*-value (Kruskal-Wallis test)
	Underweight	Normal weight	Overweight	Obese	
	(N=19)	(N=52)	(N=17)	(N=1)	
Tail Length	5.59 ± 4.01	5.97 ± 3.13	5.92 ± 3.59	4.20	0.737
Tail Moment	1.00 ± 1.64	0.84 ± 0.81	0.70 ± 0.60	0.26	0.338
Olive Moment	0.74 ± 0.68	0.72 ± 0.39	0.64 ± 0.28	0.37	0.310
% of DNA in tail	5.03 ± 3.16	5.13 ± 2.17	4.63 ± 1.24	3.11	0.235
Female (N)	16	36	14	0	
Tail Length	5.82 ± 4.18	6.01 ± 3.47	6.21 ± 3.91	-	0.871
Tail Moment	1.10 ± 1.76	0.93 ± 0.90	0.73 ± 0.63	-	0.560
Olive Moment	0.77 ± 0.73	0.75 ± 0.43	0.65 ± 0.30	-	0.537
% of DNA in tail	5.08 ± 3.30	5.21 ± 2.49	4.57 ± 1.33	-	0.480
Male (N)	3	16	3	1	
Tail Length	4.31 ± 3.26	5.88 ± 2.28	4.61 ± 1.04	4.20	0.525
Tail Moment	0.49 ± 0.54	0.64 ± 0.55	0.51 ± 0.33	0.26	0.465
Olive Moment	0.58 ± 0.41	0.64 ± 0.25	0.60 ± 0.18	0.37	0.383
% of DNA in tail	4.76 ± 2.86	4.94 ± 1.24	4.87 ± 0.80	3.11	0.463

Correlation between anthropometry data and DNA damage

The correlation between the anthropometrics data and DNA damage (tail length) was measured using Spearman's correlation test. BMI, WHR, subcutaneous fat and visceral fat showed no statistically correlation with the DNA damage ($p>0.05$, Spearman's correlation test).

DISCUSSION

This study addressed the level of oxidative DNA damage and obesity status among the Faculty of Pharmacy students. Ther overall findings showed no significant differences ($p>0.05$) in the tail length, tail moment, olive moment, and the percentage of DNA in tail were observed between groups (Table II), although greater value of DNA damage parameters (mean ± SD) was seen in overweight group. There was also no correlation between the anthropometric measurements and DNA damage ($p>0.05$) (Table III). Therefore, no significant difference in the levels of DNA damage among the normal and overweight/ obese group with no correlation between DNA damage and BMI were seen.

In the 21st century, obesity is the biggest health burden. It is a chronic condition that affects the physiological, economic, and psychological quality of an overweight/ obese patient's life compared to a normal weight patient regardless of cultural, financial, or ethnic profile (21). From a health perspective, obesity contributes to a wide range of health problems or also known as no communicable diseases, including type 2 diabetes mellitus, cardiovascular and kidney disease, as well as cancer (22). It is a multifactorial metabolic illness with complex aetiology. The impact of oxidative stress in the pathogenesis of obesity and its related risk factors has been established in epidemiological, clinical, and animal investigations (23). For example, oxidative stress is the major cause of DNA damage and if the DNA damage cannot be repaired due to several factors it can lead to various diseases. In normal weight subjects showed slightly higher value in the DNA damage parameters compared with those obese, overweight and underweight subjects. However, the tail length, tail moment, olive moment and % of DNA in the tail of obese subjects is low compared to underweight, normal weight and overweight. Thus, the results showed no significant difference between those groups and the DNA damage parameter. Next, to observe the association of BMI, with DNA damage, Spearman correlation test was used. The results also showed no correlation between those two variables. These results were contraindicated to the previous study conducted by Donmez-Altuntas et al. (24) that showed a significant increase in DNA damage lymphocytes of adult obese subjects. The study also established that there is an association between DNA damage and BMI. Besides, other studies by Al-Aubaidy and Jelinek also found a significant increment in oxidative DNA damage in obese and overweight people when compared to lean people (25).

The increasing genetic damage in obese subjects in the current study and as documented in the literature has the possibility to be caused by oxidative stress because both increased oxidizing agents and antioxidant depletion have been linked to obesity (high BMI) as well as genetic damage. This is because the increase in BMI has been linked to a decrease in essential redox regulators that are essential in scavenging ROS (7). High BMI alters the redox system by increasing the formation of ROS and lowering the activity of antioxidant defence enzymes (7). A study conducted on obese women found a decrease in the activity of SOD and GPx (45). Cellular biomolecules, such as DNA, may be adversely affected

by an imbalance in the oxidant and antioxidant status in the body (27). Other than that, a study by Karaouzene et al. (28) and Wiegand et al. (29) showed an increased level of glutathione peroxidase, superoxide dismutase and 8-hydroxy-2-deoxy guanosine in obese subjects supporting the theory of oxidative stress in obesity (27).

Other parameters that are dependent on BMI such as subcutaneous fat and visceral fat have also been observed since there's a correlation between BMI, subcutaneous fat and visceral fat. Obesity (high BMI) is associated with increased free fatty acid (FFA) circulation and excess fat deposition in visceral fat and subcutaneous fat (30). Too many plasma lipids are prone to free radical formation, which produces more ROS, and can also activate a protein kinase C pathway, resulting in increased production of nitroxide, a powerful ROS (31). Besides, the deposition of free fatty acids in adipose tissue also gives rise to ROS production by attracting leukocytes and leads to inflammation (31). However, the results also showed no relationship between adipose tissue with DNA damage. The result obtained from this study differs from a study conducted by Wlodarczyk & Nowicka (32) that reported body weight, BMI, and fat mass were all significantly correlated with DNA damage because the increase in accumulation of adipose tissue has a negative impact on DNA integrity.

WHR has also been analysed in this study in order to identify the relationship between WHR and DNA damage. However, the results also showed no significant difference between WHR and DNA damage ($P>0.05$). This result is also contraindicated with a previous study conducted which showed a positive correlation between mean frequency of DNA damage and WHR in obese women with polycystic ovary syndrome (PCOS) (33). The results obtained differ from the previous study may be due to some limitations. The first limitation is the small sample size with a lack of obese subjects. Only 1 obese subject and 19 weights subjects were volunteered in this study compared to normal weight (N=54) and underweight (N=20) subjects. The number of obese and overweight subjects may be influenced by the results obtained since this study wants to determine the association between BMI and DNA damage. The second limitation is the food habit, and the lifestyle of the subjects should be analysed. Undoubtedly, the amount of energy consumed, and the dietary pattern impact the development of obesity and obesity-related metabolic disturbances (34), as well as genome stability (35).

A study conducted by Włodarczyk et al. (32) discovered that daily intakes of energy, saturated fatty acid (SFA), and vitamins C, E, Я-carotene, and retinol influence DNA damage levels. Other examples such as a lack of folate, vitamin B, iron, and zinc, may mimic the effects of radiation on DNA, leading to the formation of oxidative lesions (36). Daily consumption of milk, milk products and high-calorific foods may also influence the production of genetic damage (27). Increased dietary fat consumption has been shown to have a major impact on DNA methylation and gene expression (37, 38). It was discovered that a high-fat diet inhibits DNA damage repair by enhancing lysine homocysteination in proteins involved in DNA damage repair (39). DNA damage also occurs due to exogenous factors because our environment contains a wide spectrum of DNA-damaging agents, such as sunlight radiation, dietary mutagens, industrial toxins, and cigarette smoke (40). The lifestyle of subjects must be analysed so that the causative of DNA damage is known. For example, smoking can effectively expose a potent chemical carcinogen to the epithelial tissue which has the potential to cause DNA damage (41). The awareness of obesity and the risk of other related diseases is important and it would help in proper action to be initiated (42).

The major limitation of this study was to recruit a sufficient sample size of subjects as research participants to achieve meaningful data. The calculation for the sample size was run however, there were only 1 obese and 17 overweight subjects. Therefore, the overall consequences of obesity-related DNA damage were not observed.

CONCLUSION

In conclusion, this study showed no association between the anthropometric measurements (BMI, WHR, subcutaneous fat, visceral fat) of subjects with DNA damage. The level of DNA damage measured in this study may be influenced by food intake and the lifestyles of subjects. The results of this study suggest that obese subjects must be included more in order to see the association between BMI and DNA damage.

ACKNOWLEDGEMENTS

A special thanks to Miss Nurul Aida (Research Assistant) and the students of Faculty Pharmacy UiTM Puncak Alam who voluntarily participated in this study. I also would like to thank Faculty of Pharmacy, UiTM for providing the facilities. This project was funded by 600-RMC/GPM ST 5/3 (001/2021). The authors acknowledge that the abstract and part of this study were presented during the International Conference on Science and Social Research 2022 (CSSR 2022).

REFERENCES

1. NHMS. National Health and Morbidity Survey (NMHS 2019): Non-Communicable Diseases, Risk Factors and Other Health Problems; Institute for Public Health: Kuala Lumpur, Malaysia, 2019.
2. Zatońska K, Psikus P, Basiak-Rasała A, et al. Obesity and Chosen Non-Communicable Diseases in PURE Poland Cohort Study. Int J Environ Res

Public Health. 2021;18(5):2701. doi:10.3390/ijerph18052701.
3. Mohd Nor NS, Chua YA, Abdul Razak S, Ismail Z, Nawawi H; MyHEBAT-CRES investigators. Identification of cardiovascular risk factors among urban and rural Malaysian youths. BMC Cardiovasc Disord. 2022;22(1):70. doi: 10.1186/s12872-021-02447-y.
4. Ruiz LD, Zuelch ML, Dimitratos SM, Scherr RE. Adolescent Obesity: Diet Quality, Psychosocial Health, and Cardiometabolic Risk Factors. Nutrients. 2019;12(1):43. doi:10.3390/nu12010043.
5. United Nation. World Youth Report: Young people's Transition to Adulthood: Progress and Challenges; United Nations Publication: New York, 2007.
6. Usman M, & Volpi EV. DNA Damage in Obesity: Initiator, promoter and predictor of cancer. Mutation research. Reviews in mutation research. 2018;778: 23–37. doi: 10.1016/j.mrrev.2018.08.002.
7. Hwalla N, & Jaafar Z. Dietary Management of Obesity: A Review of the Evidence. Diagnostics. 2020;11(1):24.doi:10.3390/diagnostics11010024.
8. Johnston BC, Kanters S, Bandayrel K, Wu P, Naji F, Siemieniuk RA, Ball GD, Busse JW, Thorlund K, Guyatt G, Jansen JP & Mills EJ. Comparison of weight loss among named diet programs in overweight and obese adults: a meta-analysis. JAMA. 2014;312(9): 923–933. doi: 10.1001/jama.2014.10397.
9. 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. International journal of molecular sciences. 2014;16(1):378–400. doi: 10.3390/ijms16010378.
10. Soares NP, Santos AC, Costa EC, Azevedo GD, Damasceno DC, Fayh AP & Lemos TM. Diet-Induced Weight Loss Reduces DNA Damage and Cardiometabolic Risk Factors in Overweight/Obese Women with Polycystic Ovary Syndrome. Annals of nutrition & metabolism. 2016:68(3): 220–227. doi: 10.1159/000444130.
11. Karaman A, Aydın H, Geçkinli B, 3etinkaya A & Karaman S. DNA damage is increased in lymphocytes of patients with metabolic syndrome. Mutation research. Genetic toxicology and environmental mutagenesis. 2015;782: 30–35. doi: 10.1016/j.mrgentox.2015.03.009.
12. Bankoglu EE, Gerber J, Kodandaraman G, Seyfried F & Stopper,H. (2020). Influence of bariatric surgery induced weight loss on oxidative DNA damage. Mutation research. Genetic toxicology and environmental mutagenesis. 2020; 853:503194. doi: 10.1016/j.mrgentox.2020.503194.
13. Merchant AT, Vatanparast H, Barlas S, Dehghan M, Shah SM, De Koning L, & Steck SE. Carbohydrate intake and overweight and obesity among healthy adults. Journal of the American Dietetic Association 2009;109(7):1165–1172. doi: 10.1016/j.jada.2009.04.002.
14. Bowden RD, Buckwalter MR, McBride JF, Johnson DA, Murray BK & O'Neill KL. Tail profile: a more accurate system for analyzing DNA damage using the Comet assay. Mutation Research. 2003;537(1):1-9. doi: 10.1016/s1383-5718(03)00056-1.
15. Fairbairn DW, Olove PL & O'Neill KL. The comet assay: a comprehensive review. Mutation Research. 1995;339:37-59. doi: 10.1016/0165-1110(94)00013-3.
16. Rojas E, Lopez MC & Valverde M. Single cell gel electrophoresis assay: methodology and applications. Journal of Chromatography. 1999;722: 225-254. doi: 10.1016/s0378-4347(98)00313-2
17. Collins AR. The comet assay for DNA damage and repair. Molecular Biotechnology. 2004;26:249-261.
18. Singh NP, McCoy MT, Tice RR & Schneider EL. A simple technique for quantification of low levels of DNA damage in individual cells. Experimental Cell Research, 1988;175:184-191. doi: 10.1016/0014-4827(88)90265-0.
19. Ahmad N, Adam SI, Nawi AM, Hassan MR & Ghazi HF. Abdominal Obesity Indicators: Waist Circumference or Waist-to-hip Ratio in Malaysian Adults Population. International journal of preventive medicine 2016;7: 82. doi: 10.4103/2008-7802.183654.
20. Shuster A, Patlas M, Pinthus JH, & Mourtzakis M. The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. The British journal of radiology. 2012;85(1009):1–10. doi: 10.1259/bjr/38447238.
21. Zhang ZY & Wang MW. Obesity, a health burden of a global nature. Acta pharmacologica Sinica. 2012; 33(2):145–147.
22. Dixon JB. The effect of obesity on health outcomes. Molecular and cellular endocrinology. 2010;316(2):104–108. doi: 10.1016/j.mce.2009.07.008.
23. Savini I, Gasperi V, Catani MV. (2016). Oxidative Stress and Obesity. In: Ahmad, S., Imam, S. (eds) Obesity. Springer, Cham.
24. 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. Mutation research. Genetic toxicology and environmental mutagenesis. 2014;771: 30–36. doi: 10.1016/j.mrgentox.2014.06.006.
25. Al-Aubaidy HA & Jelinek HF. Oxidative DNA damage and obesity in type 2 diabetes mellitus. European journal of endocrinology. 2011;164(6):899–904. doi: 10.1530/EJE-11-0053.

26. Amirkhizi F, Siassi F, Minaie S, Djalali M, Rahimi A, Chamari M. Is obesity associated with increased plasma lipid peroxidation and oxidative stress in women? [Internet]. 2010 Dec 8 [cited 2021 Dec 8];2(4). Available from: http://www.arya.mui.ac.ir/index.php/arya/article/view/165/567
27. Gandhi G. Assessment of DNA Damage in Obese Individuals. Research journal of Biology. 2015;2(2):37-44.
28. Karaouzene N, Merzouk H, Aribi M, Merzouk SA, Berrouiguet AY, Tessier C & Narce M. Effects of the association of aging and obesity on lipids, lipoproteins and oxidative stress biomarkers: a comparison of older with young men. Nutrition, metabolism, and cardiovascular diseases. 2011;21(10), 792–799. doi: 10.1016/j.numecd.2010.02.007.
29. Wiegand S, Keller KM, Rubl M, L'Allemand D, Reinehr T, Widhalm K, Holl RW & APV-Study Group and the German Competence Network Adipositas (2010). Obese boys at increased risk for nonalcoholic liver disease: evaluation of 16,390 overweight or obese children and adolescents. International journal of obesity. 2005;34(10):1468–1474. doi: 10.1038/ijo.2010.106.
30. Boden G. Fatty acids and insulin resistance. Diabetes Care. 1996;19(4):394-5.
31. Vincent HK, & Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. International journal of obesity. 2006);30(3): 400–418. doi: 10.1038/sj.ijo.0803177.
32. Włodarczyk, M., & Nowicka, G. Obesity, DNA Damage, and Development of Obesity-Related Diseases. International journal of molecular sciences. 2019;20(5):1146. doi: 10.3390/ijms20051146.
33. 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 & diseases. 2018;5(4): 367–373. doi: 10.1016/j.gendis.2018.03.001.
34. Silva Figueiredo P, Carla Inada A, Marcelino G, Maiara Lopes Cardozo C, de Cássia Freitas K, de Cássia Avellaneda Guimarres R, Pereira de Castro A, Aragro do Nascimento V & Aiko Hiane P. Fatty Acids Consumption: The Role Metabolic Aspects Involved in Obesity and Its Associated Disorders. Nutrients. 2017; 9(10):1158.
35. Ladeira C, Carolino E, Gomes MC & Brito M. Role of Macronutrients and Micronutrients in DNA Damage: Results From a Food Frequency Questionnaire. Nutrition and metabolic insights. 2017; 16(10). doi: 10.1177/1178638816684666.
36. Ames BN. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat Res Mol Mech Mutagen. 2001;475(1–2):7–20. doi: 10.1016/s0027-5107(01)00070-7.
37. Zwamborn RA, Slieker RC, Mulder PC, Zoetemelk I, Verschuren L, Suchiman HE, Toet KH, Droog S, Slagboom PE, Kooistra T, Kleemann R & Heijmans BT. Prolonged high-fat diet induces gradual and fat depot-specific DNA methylation changes in adult mice. Scientific reports. 2017;7: 43261. doi: 10.1038/srep43261.
38. Keleher MR, Zaidi R, Hicks L, Shah S, Xing X, Li D, Wang T & Cheverud JM. A high-fat diet alters genome-wide DNA methylation and gene expression in SM/J mice. BMC genomics. 2018;19(1): 888. doi: 10.1186/s12864-018-5327-0.
39. Wang D, Zhao R, Qu YY, Mei XY, Zhang X, Zhou Q, Li Y, Yang SB, Zuo ZG, Chen YM, Lin Y, Xu W, Chen C, Zhao SM & Zhao JY. Colonic Lysine Homocysteinylation Induced by High-Fat Diet Suppresses DNA Damage Repair. Cell reports. 2018;25(2):398–412.e6. doi: 10.1016/j.celrep.2018.09.022.
40. Garm C, Moreno-Villanueva M, Bürkle A, Larsen LA, Bohr VA, Christensen K & Stevnsner T. Genetic and environmental influence on DNA strand break repair: a twin study. Environmental and molecular mutagenesis. 2013;54(6):414–420. doi: 10.1002/em.21791.
41. Yamaguchi NH. Smoking, immunity, and DNA damage. Translational lung cancer research. 2019;8(Suppl 1):S3–S6. doi: 10.21037/tlcr.2019.03.02.
42. Noor Shafina MN, Abdul Rasyid A, Anis Siham ZA, Nor Izwah MK & Jamaluddin M. Parental perception of children's weight status and sociodemographic factors associated with childhood obesity. The Medical journal of Malaysia. 2020;75(3):221–225.