NJSC "WEST KAZAKHSTAN MEDICAL UNIVERSITY NAMED AFTER MARAT OSPANOV"

Abstract to the dissertation
for the degree
Doctor of Philosophy (PhD)

Analysis of DNA damage in obesity and the effect of metformin on double-stranded DNA breaks of blood lymphocytes

Specialty «6D 110100 - Medicine»

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ABSTRACT

Of Kosmuratova Raikul Nasreddinovna on the topic «Analysis of DNA damage in obesity and the effect of metformin on double-stranded DNA breaks of blood lymphocytes», presented for the degree Doctor of Philosophy (PhD) in the specialty «6D 110100 - Medicine».

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TOPICALITY

Obesity remains one of the urgent problems of modern society. The problem of obesity is acute all over the world and its further growth is predicted. In the context of the obesity pandemic and complications associated with obesity, such as reproductive disorders, diseases of the gastrointestinal tract (gastrointestinal tract), musculoskeletal system, pathology of the cardiovascular system and many types of cancer, a whole generation has grown up. At the present stage, obesity is a chronic recurrent and progressive disease, which dictates the need to take urgent measures to prevent and control this global epidemic.

Obesity worsens the course of many chronic diseases and reduces the life expectancy of the population. According to a number of scientists, obesity increases the risk of death from heart disease by 4 times and cancer pathology by 2 times. In this regard, the improvement of diagnostic markers of obesity remains an urgent task of medicine. At the same time, a personalized approach is needed not only in matters of diagnosis, but also in the development of preventive measures against complications of overweight and obesity.

Based on the above, of particular interest is the analysis of the relationship between body mass index (BMI) and damage to deoxyribonucleic acid (DNA). These studies are a new level of study of obesity, the results of which are necessary for the development of a personalized approach to the diagnosis and treatment of this pathology.

There is no consensus in the available literature on the relationship between overweight and DNA damage. But there are isolated research results that prove the connection of genetic instability in people with excess BM and obesity. Currently, the use of molecular genetic methods in diagnostics is widely used. However, regarding obesity, there is no consensus on which values of increased body weight can lead to genome instability.

The issue of finding markers for early detection of DNA damage and the possible risk of neoplasms remains relevant. At the same time, it is known that phosphorylation of the Ser-139 residue of the histone variant H_2AX is an early cellular response to the induction of double-stranded DNA breaks. Detection of this event is a highly specific and sensitive molecular marker for monitoring the initiation and resolution of DNA damage.

In addition, the analysis of H_2AX foci has many other applications in laboratory diagnostics, for example, cancer and aging research, quantitative determination of H_2AX foci is also used as a useful tool for evaluating the effectiveness of various drugs.

Thus, to understand the role of the influence of BMI on DNA damage of blood lymphocytes, it will be interesting to study double-stranded DNA breaks, as well as to analyze DNA breaks when using metformin in obese patients.

The possibility of identifying a risk group for the development of epigenetic mechanisms, determining the level of double-stranded DNA breaks will significantly increase the percentage of predicting the development of genome instability, and further development of oncopathology. And this, in turn, will increase the effectiveness of therapy and allow timely prevention of carcinogenesis in obese patients.

Such studies have not been conducted in the Republic of Kazakhstan, so there is a need to study this problem.

The purpose of our research was to study the relationship of obesity with double-stranded DNA breaks of blood lymphocytes and to analyze the effect of metformin on DNA damage.

To achieve this, the following tasks were set

1. To conduct a comprehensive clinical and laboratory assessment of overweight and obesity in the population of Western Kazakhstan.
2. To study the level of DNA damage of blood lymphocytes depending on the body mass index.
3. To analyze DNA breaks of blood lymphocytes when using metformin in obese patients.

Scientific novelty:

1. For the first time in the framework of the study, the relationship of leptin and BMI in the adult population of the Western region of Kazakhstan was studied.
2. For the first time, the level of DNA damage of blood lymphocytes was studied taking into account the body mass index.
3. For the first time, the analysis of DNA breaks of lymphocytes was performed when metformin was used in obese patients.
4. For the first time, risk factors affecting DNA instability in overweight and obesity have been identified.

Practical significance

The results of a comprehensive study of scientific data on the relationship of lymphocyte DNA damage and BMI can be used for a personalized early approach to the diagnosis of genome integrity.

Monitoring of leptin and insulin resistance can be an important part of improving diagnostics for early detection of the risk of precancerous changes in obese patients.

The results of the study can be used by government agencies in the development of standards and treatment protocols for the prediction of adverse outcomes and complications in obese patients.

The main statements made for the defense:

1. The incidence of overweight in adults in the Western region of Kazakhstan was 48%, with women being more often obese (1.7 times) and overweight (2.4 times).
2. Reliable correlation between leptin concentration and BMI, with insulin level and NOMA index was revealed.
3. the decision tree analysis of DNA damage risks showed that the presence of one of the key factors "BMI ≥ 39.4" and "Waist/Growth Index ≥ 0.7" increases the absolute risk level for the indicator "Cells with rupture foci". For the indicator "Average number of foci per cell," the three key statistically significant risk factors are "BMI, kg/m2 ≥ 39.4" and "HbA1C-Glycated Hemoglobin, % ≥ 5.6"
4. Oral administration of metformin at a dose of 850 mg/day for 3 months in obese patients (BMI greater than 30 kg/m2) leads to a decrease in the average number of DNA breaks per cell and an increase in reparation.

Approbation of the work. The main provisions of the dissertation were reported at an extended meeting of the Scientific Problem Commission of "ZKMU named after Marat Ospanov". The results of the conducted research were reported at scientific and practical conferences:

- The III international scientific and educational conference//Minerva medica//Aktobe, Kazakhstan, April 25-26, 2019.
-International scientific and practical conference "From scientific ideas to team projects" (Aktobe,October 25-26, 2019), ZKMU named after M.Ospanov.
- Materials of the International Conference "Science and innovations 2021: development directions and priorities" Australia, Melbourne, July 7, 2021.
- VI International Scientific and Practical Conference of Caspian states "Actual problems of modern medicine" (Astrakhan, October, 7-8, 2021).
- LXI International scientific-practical conference of young scientists "Science: yesterday, today, tomorrow", dedicated to 65th anniversary of ZKMU named after Marat Ospanov (Aktobe, April 27, 2022).

Publications on the topic of the dissertation. On the dissertation theme were published 10 scientific works, including 4 articles in journals indexed in the Scopus information base; 2 articles in the journals recommended by the Committee for Control in Education and Science of Kazakhstan; 1 article in the national information-analytical system; 3 theses - in the collection of International Scientific and Practical Conferences. All publications are written by doctoral students personally under the guidance of supervisors, who advised and made adjustments. Directly the doctoral student conducted the search and review of literary sources, enrollment of patients in the study group. Statistical processing and analysis of the results were performed by the doctoral student.

The study was conducted within the framework of:

1. STP "Development of scientific and methodological bases of minimization of ecological burden, medical support, social protection and recovery of the population of environmentally unfriendly territories of the Republic of Kazakhstan", funded by the Ministry of Health of the Republic of Kazakhstan for 2017 - 2019. Registration number: 0117RK00026.
2. Intramural grant registration number 0119RKI0255 on "Molecular and genetic aspects of obesity in the Kazakh population" 2019-2021.

Implementation of research results.
1) The results of the study are implemented in the practice of the department of endocrinology AMC and urban polyclinics Aktobe ;
2) Intellectual property - 2 certificates of authorship;
3) The results of the study are introduced in the curriculum of the residency at the Department of IVB №1 on specialty: "Adult, Children's Endocrinology" of ZKMU named after Marat Ospanov. The main provisions of the dissertation work are used in the lecture material and practical classes.

Author's personal contribution. Development of goals and objectives of scientific research, collection and coordination of clinical, laboratory, molecular research data, statistical processing of research results, formulation of conclusions and recommendations.

Structure and scope of work. The thesis consists of a table of contents, a list of abbreviations and definitions, an introduction, a literature review, materials and methods of research, the results of our own research, and a conclusion. The thesis is presented on 107 pages; it is illustrated with 42 tables and 53 figures. The list of references contains 196 sources; 186 of them are in English.

MATERIALS AND METHODS OF RESEARCH.

Characteristics of the study.

The research work was approved by the Bioethical Committee at the West Kazakhstan Medical University named after Marat Ospanov (Protocol No. 17 of 09.04.2019).

Research design

Stage 1: Clinical and laboratory characteristics of overweight and obesity in the adult population of the Western region of Kazakhstan (A one-stage cross-sectional study).

This stage of the study was carried out within the framework of the scientific and methodological framework for minimizing the environmental burden, medical care, social protection and health improvement of the population of environmentally unfavorable territories of the Republic of Kazakhstan, funded by the Ministry of Health of the Republic of Kazakhstan for 2017-2019. (registration number: 0117RK00026).

The study was conducted on the territory of Western Kazakhstan (Aktobe and West Kazakhstan regions). Inclusion criteria: age of participants - from 18 years; signed informed consent to participate in a scientific study. Exclusion criteria: a history of endocrine diseases (diabetes mellitus, thyroid and adrenal gland diseases), chronic decompensated diseases of internal organs, pregnancy, lactation. The recruitment of patients was carried out by random sampling, taking into account the age and gender composition of the population in public places. The number of participants in the study was 1200 people.

Stage 2: Obesity and DNA damage

The study was carried out in accordance with the Helsinki Declaration within the framework of an intra-university grant, registration number 0119RKI0255 on the topic "Molecular genetic aspects of obesity in the Kazakh population". Approved by the Bioethical Commission of the WKMU named after Marat Ospanov No. 17 dated 09.04.2019. The study was conducted in two stages.

2.1 Investigation of the level of DNA damage of blood lymphocytes depending on the BMI index (one-stage descriptive study).

The recruitment of patients to the study was carried out in Aktobe. The sample was formed taking into account the age and sex composition of the population of Aktobe. The sample size was 236 patients aged 18-60 years, the participants signed an informed consent to participate in the study. Among the participants were excluded persons with chronic decompensated diseases, pregnancy, with bad habits (smokers, drug users, alcohol).

The study of the relationship of obesity with double-stranded DNA breaks of lymphocytes. The recruitment of patients was carried out by random sampling, taking into account the age and gender composition of the population in public places. Volunteers (residents) took part in the selection for the study, who invited patients to the study. In order to ensure the representativeness of the general

population, the sample size was calculated with a confidence interval of 95% (α=5%). The sample size was 236 people.

According to the parameters of height and weight of the study participants, BMI was calculated according to the formula: weight (kg)/growth in m2, according to the WHO classification.

The participants were divided into 5 groups: group 1 – control with a BMI of 18.5-24.9 kg/m2 - normal weight, group 2 – overweight with a BMI of 25.0-29.9 kg/m2, group 3 - with a BMI of 30.0-34.9 kg/m2 - obesity of the 1st degree, group 4 - BMI of 35.0-39.9 kg/m2 - obesity of the 2nd degree, group 5 with a BMI ≥ 40kg/m2 - obesity of the 3rd degree, in which age and gender matching was carried out (Picture 2).

In each of the five groups, cells with rupture foci, foci in general, the diameter of cell ruptures, the average number of γ-H2AX foci per cell, the intensity of staining, the percentage of positive cells through two channels were analyzed: FITC ruptures and APC repair.

2.2 Analysis of DNA breaks of lymphocytes when using metformin in obese patients (prospective study).

Voluntary consent to participate in the study was obtained from 33 obese patients aged 18-60 years. During the follow-up, six subjects refused to participate further for various reasons (poor tolerance of the drug, did not show up for a repeat visit, refusal without explanation, 1 participant due to pregnancy). No serious adverse events were observed when taking the drug. As a result, 27 patients completed the study. Among the participants were excluded persons with chronic decompensated diseases, with bad habits (smokers, drug users, alcohol), pregnant women, women in the lactation period. Studies of indicators of DNA damage of lymphocytes were conducted on the basis of the Scientific and Practical Center (SPC) of the WKMU named after Marat Ospanov.

In order to study the dynamics of double-stranded DNA breaks in blood lymphocytes, patients were prescribed metformin. Obese study participants took the drug metformin in a daily dose of 850 mg for 3 months. These individuals were analyzed in the dynamics of DNA breaks of lymphocytes before and after taking metformin.

Clinical and biochemical methods of research

During the anthropometry, the following parameters were included: weight (kg), height (m), BMI kg/m2, waist (cm), hip (cm).

Blood sampling for laboratory tests was carried out in the morning on an empty stomach. The concentration of leptin ng/ml in blood serum was determined using the ELISA method - solid-phase enzyme immunoassay. Assessment of lipid status: total cholesterol was determined by the enzymatic (CHOD-PAP) method. LDL levels were calculated using Friedwald's formula (using the concentration of total cholesterol, high-density lipoproteins and triglycerides). The colorimetric method was used to study HDL. When assessing the lipid profile data, we were guided by the Recommendations of NCEP/ATPIII experts: the level of total

cholesterol>5.2mmol/l was taken for hypercholesterolemia, the level of TG>1.7mmol/l was attributed to hypertriglyceridemia. The atherogenicity coefficient (CA) was determined according to the following formula: CA = (TC–HDL):HDL.

Fasting blood glucose was determined after 12 hours of fasting. Glucose was determined by photometric method (reference values 3.89-5.83), the results were estimated in mmol/L. The level of insulin in fasting blood plasma was determined by immunoassay (reference values 2.7-29.1) while observing internal and external quality control. The results were evaluated in microns/ml. The insulin resistance index (IR HOMA) was calculated using the formula: IR HOMA = fasting glucose × fasting insulin /22.5. Normally, the HOMA index does not exceed 2.7.

Analysis of double-stranded DNA breaks of blood lymphocytes.

In order to analyze DNA breaks in lymphocytes, indirect immunofluorescence analysis was used to quantify phosphorylated γ-H2AX (Ser 139) and 53BP1 on the AKLIDES apparatus (Germany). To evaluate the results, an automatic analysis of the object's media is carried out using the AKLIDES ® Nuk system and the corresponding software. The analysis is performed by counting 100 cells at the application site using software, the following indicators were evaluated: cells with rupture foci, foci in general, cell rupture diameters, average number of gamma-H2AX foci per cell, staining intensity, percentage of positive cells.

Methods of statistical processing of research results

Descriptive statistics of quantitative variables are presented in the form of "M ± S" - an average with a standard deviation. The nonparametric Mann-Whitney criterion was used to compare two groups by numerical variables. Three or more groups were compared using the Kraskel-Wallis method. Binary and nominal values were compared between the groups according to Pearson's Chi-squared criterion. Spearman's method (rank correlation) was used to analyze correlations.

A classification tree was used to build forecasting models for key binary indicators. The method allows you to estimate the probability of target events depending on the corresponding levels of independent factors, while segmenting respondents into risk classes. The quality of the obtained models was evaluated using ROC analysis. The level of statistical confidence was considered at 0.05. Statistical data processing was performed using Statistic 10 application software packages.

RESEARCH RESULTS

Clinical and laboratory characteristics of excessive BM and obesity in the adult population of the Western region of Kazakhstan

The study was conducted on the territory of Western Kazakhstan (Aktobe and West Kazakhstan regions). Inclusion criteria: adults over the age of 18; documented consent to participate in the study.

Exclusion criteria: a history of endocrine diseases (diabetes mellitus, thyroid and adrenal gland diseases), chronic decompensated diseases of internal organs, pregnancy, lactation. The recruitment of patients was carried out by random sampling, taking into account the age and gender composition of the population in public places. The total number of participants in the study was 1200 people.

In our study, the frequency of overweight in the Western region of Kazakhstan in adults was 48%, of which 25% is overweight and 23% is obese. Women are more likely to be obese (1.7 times) and overweight (2.4 times).

The analysis of the prevalence of obesity and body fat among men and women showed significant differences between the indicators. Excess BM (70.37±3.16%) ($p<0.001$;$t=7.02$) and obesity (63.04±3.65%) ($p<0.001$;$t=4.33$) were observed in the majority of females, compared with males (29.63±4.86% and 36.96±4.77%, respectively).

The greatest difference is noted in the frequency of overweight ($p< 0.001$; $t=11.86$) and obesity of 1 st. ($p< 0.001$; $t=5.98$). With obesity of the 2nd degree, the difference between the indicators of women and men decreases. Most often, women suffered from obesity and overweight, twice as often with obesity ($p<0.001$; $t=4.33$) and 3 times more with overweight ($p<0.001$; $t=7.02$).

At the age of 18-60, the incidence of obesity is up to 12.88% among men and up to 23.88% among women. But in the 18-20-year-old group, the prevalence is higher in men, then in the 20-30-year-old age group it exceeds in women, reaching a peak of differences in 40-50-60 years ($p<0.001$, $t=3.52$; $p<0.05$, $t=2.27$). The prevalence of obesity among the female population is one and a half times higher than among men.

Next, an analysis of the blood lipid level was carried out depending on the weight of the subjects. When analyzing the data, the content of TC, TG, LDL, atherogenicity coefficient - there is a linear increase with the growth of BM, except for HDL, where there is a natural decrease in indicators.

The concentration of leptin in the blood serum, depending on BMI

In our study, the assessment of circulating leptin in the blood was carried out taking into account the body mass index, as well as data from the lipid spectrum and carbohydrate metabolism. The characteristics of the examined study participants and provides biochemical blood parameters.

Analysis of the relationship of leptin concentration with lipid profile, carbohydrate metabolism and anthropometric data using Spearman correlation coefficient showed that there is a positive relationship with BMI (p=0.57**, p<0.01), with insulin level (p=0.28**, p <0.01) and the HOMA index (p=0.21*, p <0.05). The level of leptin is highest in obesity of the 3rd degree.

The analysis of the serum leptin content, depending on age, has no significant differences between the groups (df=3; p=2.32).

The analysis of the values of the serum leptin content showed significant differences by gender. In women, it was 24.20 ng/ml, in men - 12.89 ng/ml. In our study, a statistically significant difference in serum leptin concentration was found in the study group of women in relation to the male group (P< 0.0001).

Similar gender differences were found in other studies, while a positive correlation of leptin levels with BMI was also found in the examined both sexes. Gender differences are apparently due to the influence of sex hormones involved in the control of leptin secretion and having a decisive influence on the activity of neurohumoral adipose tissue.

Analysis of the relationship of leptin concentration with lipid profile, carbohydrate metabolism and anthropometric data using Spearman correlation coefficient showed that there is a positive relationship with BMI (p=0.57**, p<0.01), with insulin level (p=0.28**, p <0.01) and the HOMA index (p=0.21*, p <0.05).

Thus, in our work, the serum leptin content correlates with BMI, the highest rates were observed at a BMI of 40 kg/m2; a direct relationship of leptin with insulin and the HOMA index was revealed.

To study the level of DNA damage of blood lymphocytes depending on BMI

The study of the relationship of obesity with double-stranded DNA breaks of lymphocytes. At this stage of the study, the total number of subjects was 236 participants (one patient had blood hemolysis). To study the level of DNA damage of blood lymphocytes, depending on the body mass index, the following indicators were analyzed: "Cells with foci of ruptures", "Foci in general", "Diameter of cell ruptures", "Average number of foci per cell", "Intensity of glow", "Percentage of damaged cells".

DNA damage depending on BMI along the FITC channel – breaks. DNA damage as a function of BMI according to the gap channel showed that 5 out of 6 indicators differed statistically significantly between the five compared groups. The most significant differences were found for the indicator "Average number of foci per cell, n" in the "30.0-34.9" group relative to the "18.5-24.9" group (average of 1.6 n; P < 0.0001); "Focuses overall, n" index in the "40.0 or more" group relative to the "18.5-24.9" group (mean by 30.9 n; P < 0.0001); "Cells with gap foci, n" index in the "40.0 or more" group relative to the "35.0-39.9" group (mean by 27.1 n; P < 0.0001).

DNA damage as a function of BMI by repair channel 1 of 6 indicators was statistically significantly different between the five compared groups. A statistically significant difference was found for the luminescence intensity, AU indicator in the "40.0 or more" group relative to the "30.0-34.9" group (mean of 173.6 AU; $P < 0.0001$). The most homogeneous distributions between the five groups were observed for the indices: "cell rupture diameter, μm" ($P > 0.7831$).

To present changes in the mean value of the DNA break indices in the studied groups, we plotted the percentages of the break channel and reparation channel indices. According to the dynamics, there was an increase in the index "Cells with gap foci" in the studied groups, and only in group 5 there was a significant increase in the number of cells with DNA breaks by 2.9 times compared to the control group, while the reparation level changed insignificantly and did not cover the number of DNA breaks.

There was an increase in the indicator "Average number of foci per cell" in the groups, and only in persons with the 3rd degree of obesity there was a significant increase in the number of cells with DNA breaks by 4.6 times compared to the control group, while the reparation level changed insignificantly and did not restore DNA reparation.

Influence of age and gender on DNA damage

The analysis of the interrelationships of DNA damage and age was carried out. Median rupture diameters (Me [LQ; UQ]) under the age of 29.9 years was 0.49 [0.382;0.612] μm; 30-39.9 years - 0.481 [0.429;0.542] μm; 40-49.9 years – 0.452 [0.377;0.565] μm; over 50 years – 0.489 [0.448;0.563] μm. There were no significant differences between the age categories (Kruskal-Wallis test: H =2.61; p =,4557).

The average number of ruptures per cell under the age of 29.9 years was 0,020 [0,009;0,902]; 30-39,9 years - 0,014 [0,016;0,800]; 40-49,9 years – 0.042 [0.007;0.546]; more than 50 years – 0.049 [0.009; 1.016]. The median test revealed a significant difference between different age groups (Chi-Square = 10.39; p = ,0155).

The greatest differences are observed in the second group (overweight), 16.9% among women and 26.8% among men. Sex-adjusted comparisons for all six indicators: cells with rupture foci, foci in general, the diameter of cell ruptures along the rupture channel.

Comparison by gender of DNA damage indicators along the rupture channel. (Mann-WhitneyTest). We found the most significant differences by sex, with women having a 1.3-fold ($P = 0.0252$) higher rate of "Cells with rupture foci"; a 30% ($P = 0.0251$) rate of "Average number of ruptures per cell"; a 26% ($P = 0.0203$) rate of "percent damaged cells" compared to men. This seems to be related to a higher frequency of hormonal, metabolic abnormalities in women.

Formation of risk classes for the development of the indicator "Cells with foci of ruptures"

One of the objectives of the study is the ability to quickly carry out rapid diagnosis of new patients. For this purpose, all patients are divided into several risk classes of the target event using a combination of influencing factors and, further, the classes are ranked according to the level of risk. To solve this problem, the method of classification trees has proven itself well.

To assess the predictive quality of the constructed decision tree, such indicators as AuROC, sensitivity and specificity are used. If the AuROC value is less than 0.75, then the predictive qualities of the tree are at a low level, with values greater than 0.75, the predictive quality is at an average level and values above 0.85 indicate a high predictive quality of the model.

Based on the results of a one-factor risk analysis of the target indicator "Cells with rupture foci", it can be concluded that the three key statistically significant risk factors are "BMI, kg/m2 ≥ 39.4", "BMI groups (From 40.0 and more)" and "Waist/height index ≥ 0.6" with absolute risk levels more than 42.1%. The presence of one of the three key factors increases the risk level of this indicator by more than 2.4 times.

Graphically shows the odds ratio (95% CI) of statistically significant factors affecting the target indicator "Average number of foci per cell"

For the target indicator "Average number of foci per cell", the three key statistically significant risk factors are For the target indicator "Average number of foci per cell", the three key statistically significant risk factors are "BMI, kg/m2 ≥ 39.4" and "HbA1c-Glycated hemoglobin, % ≥ 5.6" with levels of the absolute risk is up to 80%.

Analysis of DNA breaks of blood lymphocytes when using metformin in obese individuals

At this stage of the study, a pilot study was conducted on the effect of metformin on the level of DNA breaks of lymphocytes before and after use in obese individuals. The analysis was carried out using the Wilcoxon criterion, since 2 time periods are allocated in the study. The main task of the method is to determine whether or not there were statistically significant changes in the indicator between measurements in the direction of increasing or decreasing.

27 patients participated in the study. In order to study the dynamics of DNA breaks in blood lymphocytes, patients were prescribed the oral drug Metformin at a daily dose of 850 mg / day for 3 months. When taking the drug, no undesirable manifestations were observed.

The analysis of double-stranded DNA damage before and after the use of metformin was carried out.

Analysis of the dynamics of indicators before and after taking metformin. The index of the average number of breaks per cell was 0.41±0.010 before

metformin administration, after metformin administration decreased to 0.30±0.086 (by 26.82%) ($p<0.0001$).

Analysis of the dynamics of quantitative indicators on the repair channel before and after metformin administration. After metformin administration there was a significant significant increase in the level of reparation according to the indicator "Average number of reparation per one cell" by 47,5% compared to before the drug administration.

Based on the results obtained, we can conclude that during the time period under consideration all indicators do not change statistically significantly in the reparation channel, except for the indicator of the average number of reparations per one cell. Apparently, the increase in DNA strand repair during metformin administration is associated with the activation of intracellular checkpoints of response to DNA damage and suppression of cellular immortalization.

CONCLUSION

Thus, the following conclusions were made based on the results of our study:

1. The frequency of overweight in the Western region of Kazakhstan in adults was 48%, of which 25% is overweight and 23% is obese. Women are more likely to be obese (1.7 times) and overweight (2.4 times).
2. There was a significant positive association of leptin concentration with BMI ($p=0.57$, $p<0.01$), with insulin level ($p=0.28$, $p<0.01$) and the HOMA index ($p=0.21$, $p<0.05$).
3. An increase in DNA damage in obesity of the 3rd degree was proved by 2.9 times in terms of "Cells with rupture foci" and by 4.6 times in terms of "Average number of foci per cell" compared to the control group.
4. Analysis of the risks of DNA damage to the decision tree showed that the presence of one of the key factors "BMI ≥ 39.4" and "Waist/height index ≥ 0.7" increases the level of absolute risk to 84.6% according to the indicator "Cells with rupture foci". For the target indicator "Average number of foci per cell", the three key statistically significant risk factors are "BMI, kg/m2 ≥ 39.4" and "HbA1c-Glycated hemoglobin, % ≥ 5.6" with absolute risk levels up to 80%.
5. Oral administration of metformin at a dose of 850 mg / day for 3 months in obese patients (BMI more than 30 kg / m2) leads to a decrease in the average number of DNA breaks per cell by 26.8% and an increase in the level of repair by 47.5%.

PRACTICAL RECOMMENDATIONS

General practitioners, therapists, endocrinologists, in addition to the main diagnostic criteria for a comprehensive examination of obese patients, it is recommended to determine the level of leptin, insulin, insulin resistance index (HOMA index), since the combination with "BMI ≥ 39.4" and "Waist / height index ≥ 0.7" leads to an increased risk of DNA damage. When identifying these

indicators in patients, it is recommended to include them in the risk group with further monitoring in dynamics in order to prevent the development of precancerous conditions.