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Results of construction of correlation fields with trend lines and determination of direction of relationships between studied indicators

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Abstract

The aim of work is construction of correlation fields with trend lines and determination of the directionality of relationships between the investigated indicators of endothelial dysfunction, hypoxia and oxidative stress.

Our results indicate that Willebrand factor, endothelin-1, 2,3-diphosphoglycerate, MDA, diene conjugates and eNOS are informative markers in the pathogenesis of experimental diabetic retinopathy. As a result of the research, it was established that there is a direct linear relationship between the Willebrand factor indicator and malondialdehyde, since the points of the correlation field are practically located on a straight line. We trace a similar type of direct linear relationship between Willebrand factor and diene conjugates. The specified results confirm the mutually aggravating relationship between the development of endothelial dysfunction and oxidative stress. We trace an inverse linear relationship between

Willebrand factor and peroxidase. With an increase in Willebrand factor indicators by stages, we trace a decrease in peroxidase indicators, which indicates a relationship between endothelial dysfunction and a weakening of antioxidant protection. As a result of the study, it was established that the first two indicators: malondialdehyde and diene conjugates have an almost direct linear relationship with endothelin-1 indicators. These results confirm the relationship between the functional and structural state of the endothelium and the development of oxidative stress. It was established that the peroxidase indicator has an inverse linear relationship with the endothelin-1 indicators, which, as in the case of the Willebrand factor, indicates a violation of antioxidant protection in the event of endothelial dysfunction. As a result of the analysis of the dependence of the indicators of 2,3 diphosphoglycerate, Willebrand factor and eNOS, it was found that in the first two pairs of indicators, there is an inverse linear relationship between the indicators. As eNOS levels increase by stage, levels of 2,3 diphosphoglycerate and Willebrand factor decrease. In the case of an inverse relationship between the parameters of the Willebrand factor and eNOS, a practically functional connection is observed, since the coefficient of determination is almost 1. The obtained results indicate that the violation of the structural and functional state of the endothelium and the development of hypoxia are interconnected with the violation of the physiological pathway of oxide synthesis nitrogen. The relationship between the indicators of 2,3 diphosphoglycerate and the Willebrand factor was revealed, which has a direct relationship between the variables, which once again confirms the aggravating interaction between hypoxia and endothelial dysfunction.

Keywords: experimental diabetic retinopathy; streptozotocin diabetes; endothelial dysfunction; oxidative stress; hypoxia; nitric oxide synthesis.

Introduction. Diabetic retinopathy (DR) is the main cause of visual impairment in the population of economically developed countries and is diagnosed in 40-85 % of patients suffering from diabetes. According to WHO data, it is the key etiological factor of vision loss and blindness in diabetes [1-4].

It is noting that even with the compensation of carbohydrate metabolism, the development of DR continues. An important factor in the development of retinopathy in diabetes is not only hyperglycemia, but also arterial hypertension [5-9]. The key role of endothelial dysfunction in the occurrence and progression of DR has been proven [10, 11]. The pathogenesis of complications of diabetes mellitus is multifactorial in nature [12, 13].

The aim of work: construction of correlation fields with trend lines and determination of the directionality of relationships between the investigated indicators of endothelial dysfunction, hypoxia and oxidative stress.

Materials and methods. The study was conducted on white Wistar rats weighing 180-200 g. According to the tasks, the animals were divided into 3 groups:

1st group – intact animals;

2nd group – 60 animals with modelling of DR without correction (control pathology).

Type 2 diabetes and DR were modeled by intraperitoneal administration of streptozotocin (Sigma, USA) dissolved in 0.1 M citrate buffer with pH 4.5 [14, 15]. Dose of streptozocin of 55 mg/kg of animal weight was divided into two administrations. Administration of streptozocin was preceded by a high-fat diet for 28 days [16].

Animals were subjected to research by decapitation in accordance with the "Rules for the performance of work using experimental animals", approved by the Order of the Ministry of Health of Ukraine No. 249 of 01.03.2012 and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (as amended on 15.12.2009 and 16.10.2012).

Calculations of logistic regression parameters and characteristics that describe the adequacy of the model were carried out in the PASW Statistics 18 statistical package. The maximum likelihood method was used as the loss function, the statistical significance of the model was assessed using several Xi-square and Hosmer-Lemeshev criteria [17]. The degree of certainty (coefficient of determination) according to Cox and Snell in our model is equal to 0.632 (63.2 %). This criterion shows the degree of influence of all factor characteristics on the variance of the dependent variable.

The situation, when the explained variable in the model takes only two different values, arises when studying the influence of certain subjective and objective factors on the presence or absence of a certain characteristic in certain individuals, households, etc. If the study covers nn subjects, i.e. if any nn observations, the fact of the presence or absence of such a feature in ii observation it is convenient to index with the numbers 1 (presence of the feature) and 0 (absence of the feature). Thus, the indicator (dichotomous, binary) variable is determined yy, which get at iiobservation value $y_i y_i$. For that $y_i = 1 y_i = 1$ if there is a sign ii of subject and $y_i = 0 y_i = 0$ – absence of sign in ii that subject [18].

The task of logistic regression is to explain the presence or absence of the characteristic under consideration by the values (more precisely, a combination of values) of

some factors (explanatory variables). Accordingly, the task is to estimate the parameters of the binary choice model, which is generally written as follows:

$$y_i = G\left(\theta_1 x_{i1} + \cdots + \theta_p x_{ip}\right) + \varepsilon_i, \qquad i = 1, \ldots, n,$$

where $x_{i1}, \ldots, x_{ip} x_{i1}, \ldots, x_{ip}$ – value pp explanatory variables in ii observation; $\theta_1, .., \theta_p \theta_1, .., \theta_p$ – unknown parameters; $\varepsilon_1, \ldots, \varepsilon_n \varepsilon_1, \ldots, \varepsilon_n$ – random errors, which reflect the influence of the presence or absence of the investigated characteristic ii in subject some unaccounted-for additional factors; $G(z) G(z)$ _ SS - similar distribution function. As a function $G(z) G(z)$ we chose the standard logistic distribution function (logit model): $\frac{e^z}{1 + e^z}$. Let's define the dependent dichotomous variable yy as «the transition of non-proliferative diabetic retinopathy to the proliferative stage», i.e., $y_i = 1 y_i = 1$ in the presence of the transition of non-proliferative diabetic retinopathy to the proliferative stage and $y_i = 0 y_i = 0$ in the absence of this fact.

The following three variables were used as factors:

1. $x_1 x_1$ - 2,3 erythrocyte diphosphoglycerate;
2. $x_2 x_2$ - endothelin-1;
3. $x_3 x_3$ - Willebrand factor.

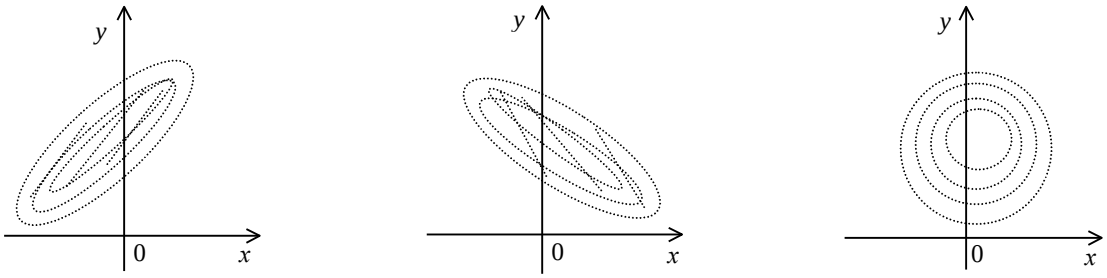
Correlation fields with trend lines were also constructed, on which there are coefficients of determination. Graphs illustrating the nature of the relationship (direct or inverse) are also constructed.

When two quantities appear together as a result of the experiment, the researcher has grounds for establishing a certain dependence between them, a connection.

Strict functional dependence between variables in the real world does not exist, as variables are influenced by random factors, the consequences of which are almost impossible to predict. Therefore, there is a special form of connection between variables, which is called stochastic, and which in mathematical statistics is transformed, without changing its essence, into statistical dependence [17, 18].

Studying two variables XX and YY changing values $X = x_i X = x_i$ led to the change of YY, which can be divided into two components: systematic, related to the dependence that exists between XX and YY, and random, which is influenced by random factors.

The formula for the dependence between variables X and Y can be established using correlation fields (Fig. 1).



a) b) c)

Fig. 1. Forms of the correlation field

Here each point with coordinates ($x_i; y_i x_i; y_i$) corresponds to a certain numerical value of the features XX and YY.

At the Fig. 1a the set of points has a trend with increasing values XX cause increase in the values of the characteristic YY. The type of communication here is direct.

At the Fig. 1b the set of points has a trend with increasing values XX cause decrease in the values of the characteristic YY. The type of communication here is reversed.

At the Fig. 1c points are evenly spaced on the coordinate plane. This indicates that there is no dependence between the features XX and YY.

Suppose that between variables XX and YY theoretically there is a certain linear dependence. Such an assumption can be made based on the analysis of the correlation field for pairs ($x_i; y_i x_i; y_i$), if it looks like it is shown in fig. 2

Between the signs XX and YY there is close connection, but not a strictly functional one. Some observed values yy will deviate from the predicted linear dependence under the influence of random factors, which are mostly unknown.

Considering the impact on value YY of random factors, linear dependence equation (linear trend, linear pairwise regression equation) XX and YY can be written in this form:

$$y_i = \beta_0 + \beta_1 x_i + \varepsilon_i ,$$

where $\beta_0\beta_0$, $\beta_1\beta_1$ – unknown regression parameters, $\varepsilon_i\varepsilon_i$ is a random variable that characterizes the deviation yy from a hypothetical theoretical regression.

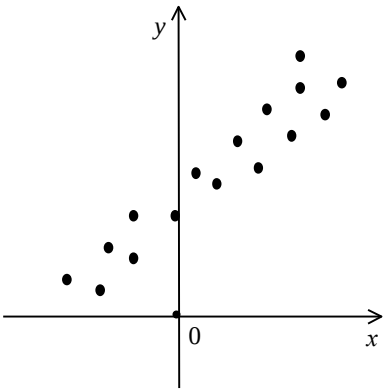


Fig. 2. Correlation field with a linear dependence between variables

Thus, we need to define the parameters $\beta_0\beta_0$, $\beta_1\,\beta_1$. But it is impossible to get the true values of these parameters, since we use information obtained from a sample of limited volume. Therefore, the obtained parameter values will be only statistical estimates of the true parameters $\beta_0\beta_0$, $\beta_1\beta_1$. Denote through $\beta_0^*\beta_0^*$, $\beta_1^*\beta_1^*$ parameter values that we got based on sample processing. Then models $y_i = \beta_0 + \beta_1 x_i + \varepsilon_i$ a statistical estimate will correspond$\hat{y}_i = \beta_0^* + \beta_1^* x_i$.

Most often the parameters $\beta_0^*\beta_0^*$, $\beta_1^*\beta_1^*$ are determined by the method of least squares, which is the basis of most statistical packages for calculating the parameters of the regression equation (trend).

According to this method, the linear pairwise regression equation $\hat{y}_i = \beta_0^* + \beta_1^* x_i\hat{y}_i = \beta_0^* + \beta_1^* x_i$ are chosen so that the sum of squared deviations of the observed values from the regression line (trend line) would be minimal.

A regression line is drawn through the correlation field $\hat{y}_i = \beta_0^* + \beta_1^* x_i\hat{y}_i = \beta_0^* + \beta_1^* x_i$. Deviation of any point with coordinates ($x_i; y_i x_i; y_i$), equal to the value $\varepsilon_i\varepsilon_i$ (Fig. 3).

The parameters are according to the following formulas:

$$\beta_0^* = \bar{y} - \beta_1^* \cdot \bar{x}$$

$$\beta_1^* = \frac{\frac{\sum x_i y_i}{n} - \bar{x}\bar{y}}{\frac{\sum x_i^2}{n} - (\bar{x})^2},$$

де $\bar{x}\bar{x}$, $\bar{y}\bar{y}$ – the average values XX and YY.

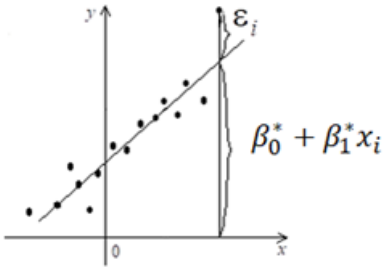


Fig. 3. Correlation field with regression line (trend line).

An important indicator of the chosen regression equation is the coefficient of determination:

$$R^2 = \frac{\sum_{i=1}^{n}(\hat{y}_i - \bar{y})^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}.$$

The coefficient of determination assesses the adequacy of the correspondence of the selected trend line with empirical data and determines what proportion of the variation of one indicator depends on the variation of another [18]. The closer it is to 1, the better the trend reflects the dependence between variables ($0 \le R^2 \le 10 \le R^2 \le 1$). If it is close to 0, then there is no dependence between the variables.

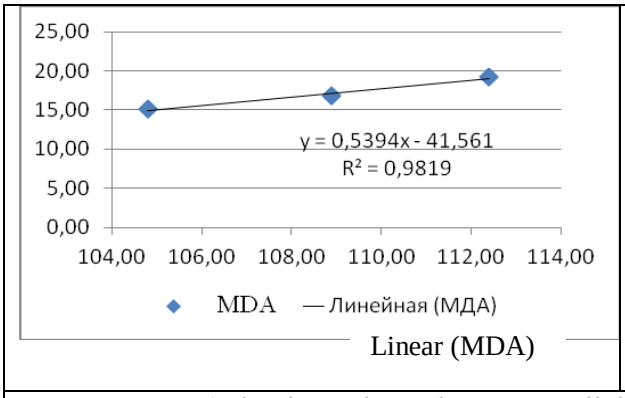
Results of study and their discussion:

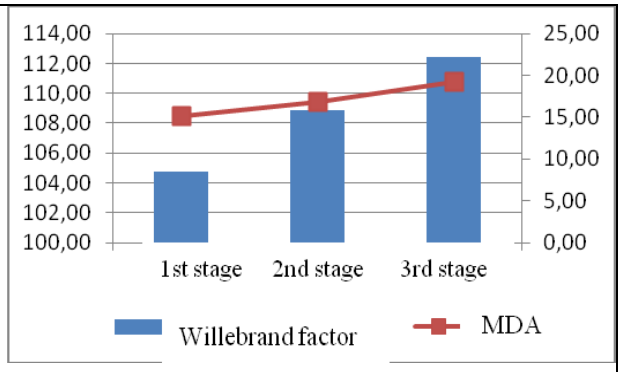
We will analyze the relationship between the average values of Willebrand Factor, Endothelin-1 and MDA, Diene conjugates, Peroxidase in group 2 (Fig. 4). Note that the number of stages is three, so the calculation of the correlation coefficient and its interpretation are not correct. But it is advisable to analyze correlation fields and analyze indicators based on graphs.

To visualize the dependence between each pair of indicators, we will build two graphs:

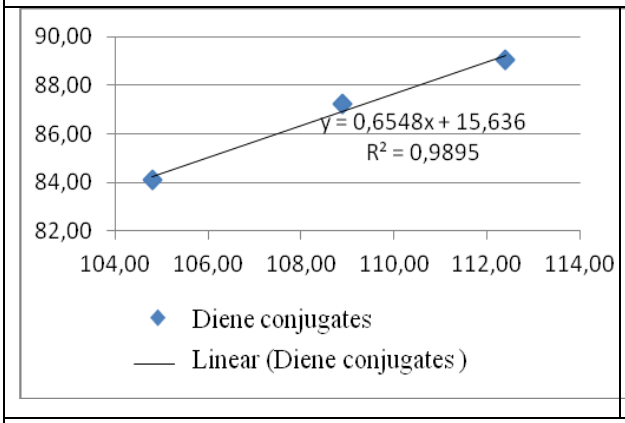
1. *Correlation field with linear trend and coefficient of determination*. Each correlation field will have three points (by the number of stages) with the coordinates of the points ($x_i; y_i x_i; y_i$), where $x_i x_i$ will determine the value of the 1st indicator, $y_i y_i$ values of second indicator on ii stage. We obtained the correlation field, linear trend, and coefficient of determination using the appropriate functions in MS Excel;

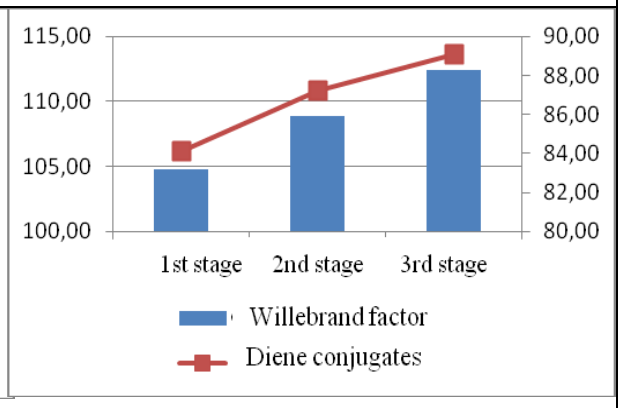
2. *A graph with the value of both indicators*, on which we will use two axes (left and right), since each pair of indicators has different units of measurement.



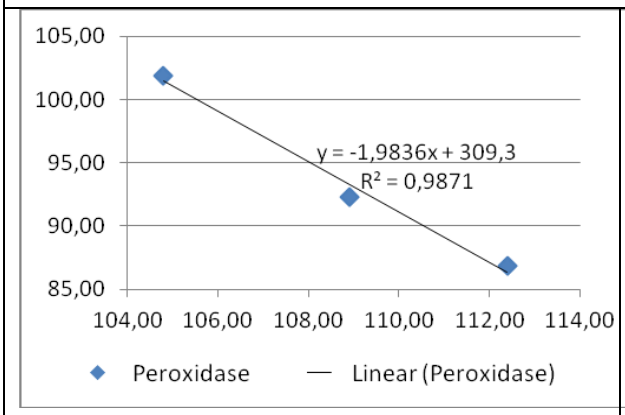


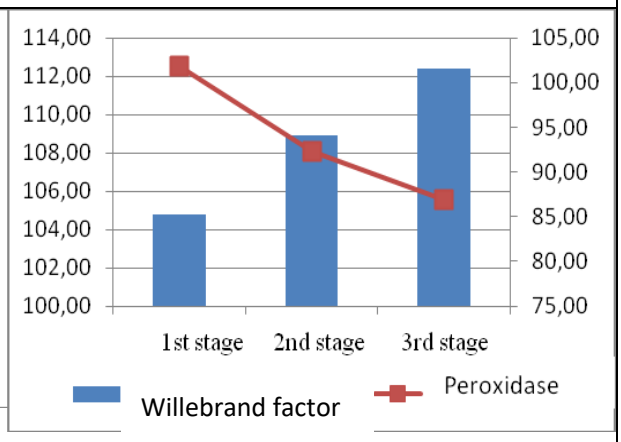
a) the dependence between Willebrand Factor and MDA indicators (Direct dependence)





b) the dependence between Willebrand factor and diene conjugates (Direct dependence)





c) dependence between Willebrand Factor and Peroxidase indicators (reverse link)

Fig. 4. Graphs of dependence between the average values of the Willebrand factor, MDA, Diene conjugates, Peroxidase

Fig. 4 shows that there is a direct linear dependence between the Willebrand factor indicator and the MDA indicator, since the points of the correlation field are practically located on a straight line ($R^2 = 0{,}982$). We trace a similar type of direct linear dependence

between the Willebrand Factor and diene conjugates ($R^2 = 0{,}99$). The specified results confirm the mutually aggravating dependence between the development of endothelial dysfunction and oxidative stress. But between the Willebrand factor and peroxidase, we trace an inverse linear relationship ($R^2 = 0{,}987$). With the increase in Willebrand factor indicators by stages, we follow a decrease in peroxidase indicators, which indicates the relationship between endothelial dysfunction and the weakening of antioxidant protection. We will similarly analyze the relationship between the average values of endothelin-1 and malondialdehyde, diene conjugates, and peroxidase in group 2.

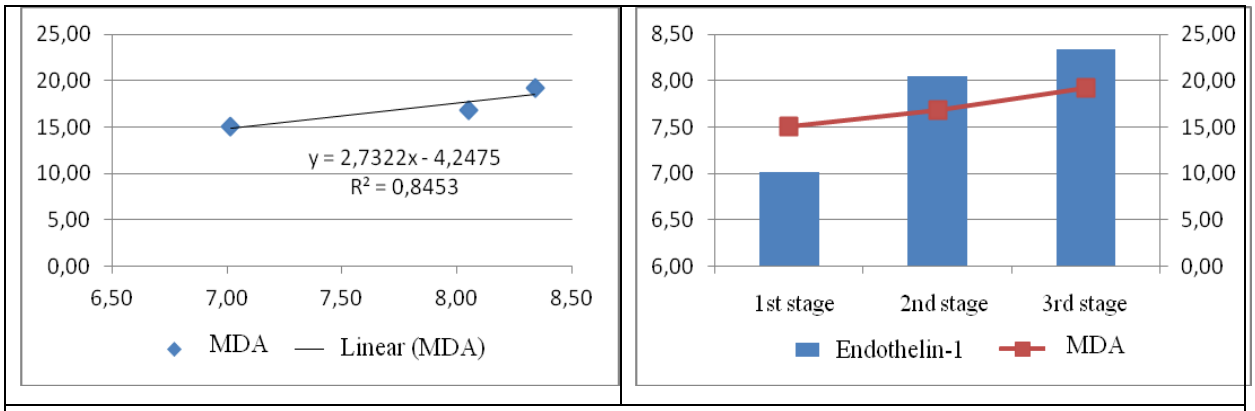
Fig. 2 shows the similar situation to the previous one. The first two indicators of malondialdehyde, diene conjugates have an almost direct linear dependence with endothelin-1 indicators a)$R^2 = 0{,}845R^2 = 0{,}845$; b) $R^2 = 0{,}973R^2 = 0{,}973$). These results confirm the dependence between the functional and structural state of the endothelium and the development of oxidative stress. And the peroxidase indicator has an inverse linear dependence with endothelin-1 ($R^2 = 0{,}976R^2 = 0{,}976$), which, as in the case of the Willebrand factor, is evidenced by a violation of antioxidant protection in the event of endothelial dysfunction.

We will analyze the dependence of indicators of 2,3 diphosphoglycerate, Willebrand factor and eNOS (Fig. 6).

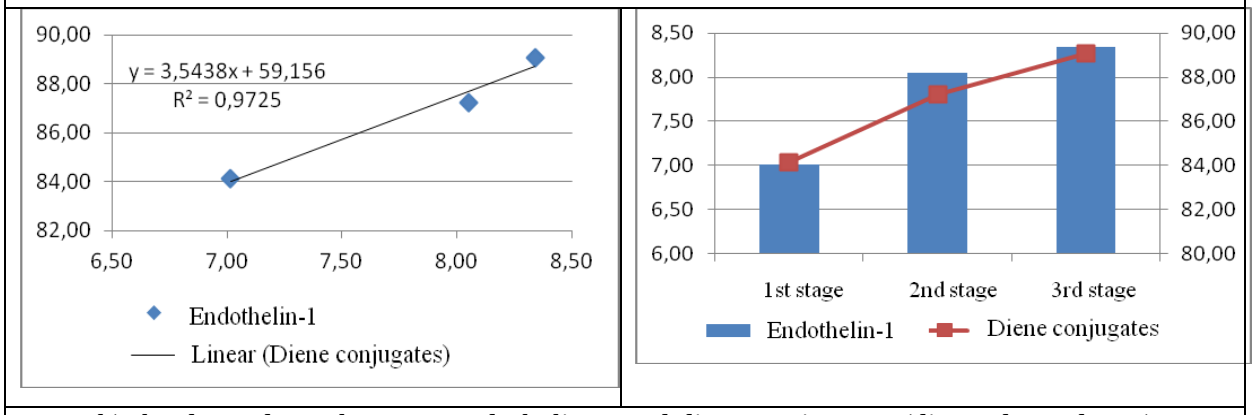
Fig. 3 shows that in the first two pairs of indicators a) and b) there is an inverse linear dependence between the indicators. As eNOS levels increase by stage, levels of 2,3-diphosphoglycerate and Willebrand factor decrease.

In the case of an inverse relationship between Willebrand factor and eNOS indicators, an almost functional relationship is observed, since the coefficient of determination is almost 1 ($R^2 = 0{,}996R^2 = 0{,}996$). The obtained results indicate that the violation of the structural and functional state of the endothelium and the development of hypoxia are interconnected with the violation of the physiological pathway of nitric oxide synthesis.

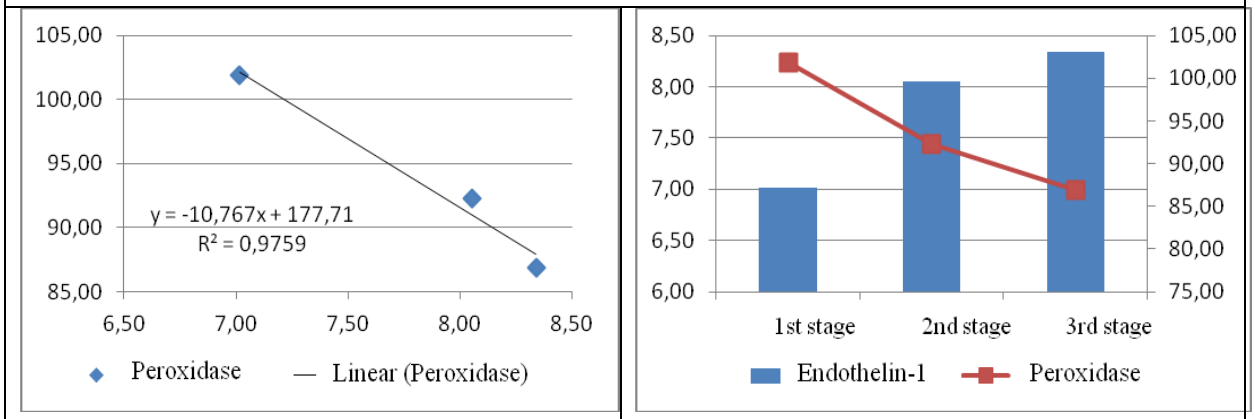
But the relationship between the indicators of 2,3-diphosphoglycerate and the Willebrand factor has a direct dependence between the variables ($R^2 = 0{,}903R^2 = 0{,}903$), which once again confirms the aggravating interaction between hypoxia and endothelial dysfunction.



a) the dependence between endothelin-1 and MDA indicators(direct dependence)

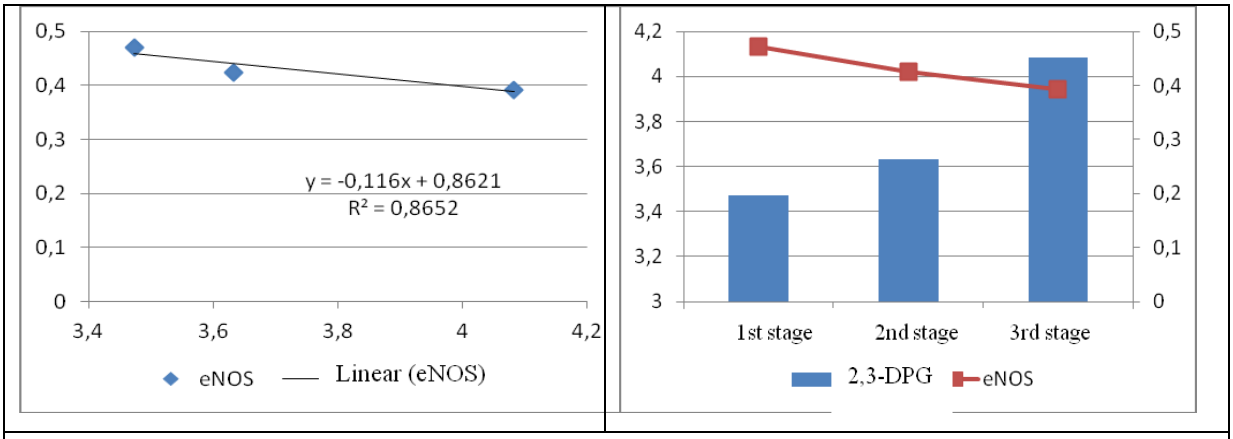


b) the dependence between endothelin-1 and diene conjugates (direct dependence)

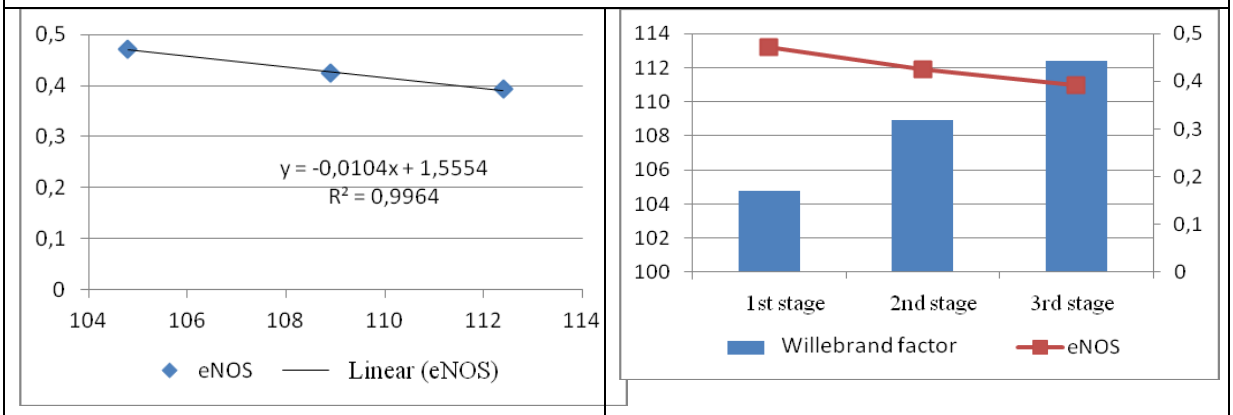


c) the dependence between endothelin-1 and peroxidase (reversed dependence)

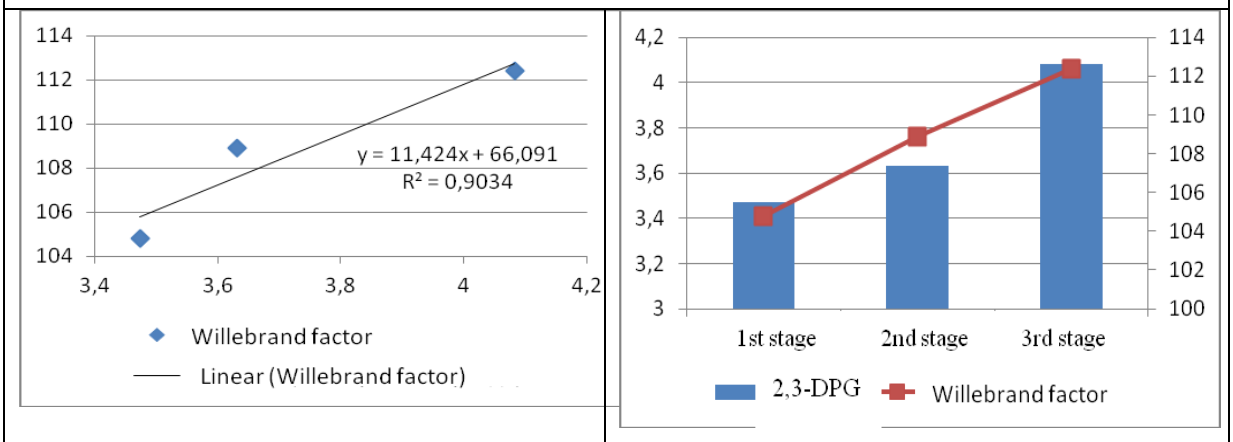
Fig. 5. Graphs of the dependence between the average values of the endothelin-1 index and the average values of the MDA, diene conjugates, and peroxidase indicators



a) the dependence between 2,3-diphosphoglycerate and eNOS indicators (reverse link)



б) the dependence between Willebrand factor and eNOS indicators (reverse link)



c) dependence between indicators of 2,3 diphosphoglycerate and Willebrand factor (direct dependence)

Fig. 6. Graphs of dependence between the mean values of 2,3 diphosphoglycerate, Willebrand factor and eNOS

All trends shown in Figures 4-6 are very well agreed with empirical data, as they are characterized by high coefficients of determination.

Conclusions:

1. Our results indicate that Willebrand factor, endothelin-1, 2,3-diphosphoglycerate, MDA, diene conjugates and eNOS are informative markers in the pathogenesis of experimental diabetic retinopathy.

2. As a result of the research, it was established that there is a direct linear relationship between the Willebrand factor indicator and malondialdehyde, since the points of the correlation field are practically located on a straight line. We trace a similar type of direct linear relationship between Willebrand factor and diene conjugates. The specified results confirm the mutually aggravating relationship between the development of endothelial dysfunction and oxidative stress.

3. We trace an inverse linear relationship between Willebrand factor and peroxidase. With an increase in Willebrand factor indicators by stages, we trace a decrease in peroxidase indicators, which indicates a relationship between endothelial dysfunction and a weakening of antioxidant protection.

4. As a result of the study, it was established that the first two indicators: malondialdehyde and diene conjugates have an almost direct linear relationship with endothelin-1 indicators. These results confirm the relationship between the functional and structural state of the endothelium and the development of oxidative stress.

5. It was established that the peroxidase indicator has an inverse linear relationship with the endothelin-1 indicators, which, as in the case of the Willebrand factor, indicates a violation of antioxidant protection in the event of endothelial dysfunction.

6. As a result of the analysis of the dependence of the indicators of 2,3 diphosphoglycerate, Willebrand factor and eNOS, it was found that in the first two pairs of indicators, there is an inverse linear relationship between the indicators. As eNOS levels increase by stage, levels of 2,3 diphosphoglycerate and Willebrand factor decrease.

7. In the case of an inverse relationship between the parameters of the Willebrand factor and eNOS, a practically functional connection is observed, since the coefficient of determination is almost 1. The obtained results indicate that the violation of the structural and functional state of the endothelium and the development of hypoxia are interconnected with the violation of the physiological pathway of oxide synthesis nitrogen

8. The relationship between the indicators of 2,3 diphosphoglycerate and the Willebrand factor was revealed, which has a direct relationship between the variables, which once again confirms the aggravating interaction between hypoxia and endothelial dysfunction.

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