APPRAISAL OF CHANGEABILITY OF THE EMN INDEX WITH REGARD TO LIFESTYLE ON THE BASIS OF DAILY EXAMINATION

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Purpose of the Study
The purpose of the study is:
♦ To show the changes in the values of the EMN index in daily measurements with the application of the two methods of cell count: repetitive method and sequential method.
♦ To evaluate the stability of the EMN values in two different individuals with regard to their life style in the period of 60 days.
♦ Additionally, measurement error resulting from differences in the methods of counting was estimated and the method better reflecting the actual biological state of a studied organism was indicated. The repeatability of the measurements was also studied.

Material and methods
The subjects were two male individuals declaring different lifestyles with regard to the following features:
♦ rational and regular alimentation,
♦ regular sleep and daily activities,
♦ well-being and little exposure to stress.

With regard to the above-mentioned characteristics one individual was marked as the positive one, since all the above-listed features were present in his lifestyle. The other individual whose lifestyle lacked the features to a certain degree was marked as a negative one. Buccal epithelium was sampled and examined according to the method proposed by [SHAKHBAZOV 1986, MAKALOWSKA 1992], if possible, on a daily basis between 12 p.m. and 2 p.m. The cases when samples were taken at a different time of the day and any other unusual events were recorded.

Every day each individual was studied with the use of two counting methods. Repetitive one consisted in the counting of up to 100 cells on the glass repeated three times, while sequential counting consisted in the counting of consecutive sequences of a 100, 200, 300 cells, where each of the results in turn was recorded.

Results
The results of the study are presented in the diagrams.
Diagram 1

Repetitive measurement taken from the individual of the positive type

Diagram 2

Sequential measurement taken from the individual of the positive type
Diagram 3
Repetitive measurement taken from the individual of the negative type

Diagram 4
Sequential measurement taken from the individual of the negative type
Diagram 5
Mean weekly EMN values for the individual of the positive type

Diagram 6
Mean weekly EMN values for the individual of the positive type
Diagram 7
Mean weekly EMN values for the individual of the negative type

Diagram 8
Mean weekly EMN values for the individual of the negative type
Diagram 9
Regression of the EMN mean - positive type - with regard to the week variable

positive type = 35,955 + 0.05808 * week
Correlation: r,01404

Diagram 10
Regression of the EMN mean - positive type - with regard to the week variable

positive type = 37,160 + 0.02219 * week
Correlation: r,00561
Diagram 11
Regression of the EMN mean - negative type - with regard to the week variable

negative type = 26,448 + 1,3719 * week

Correlation: r,34938

Diagram 12
Regression of the EMN mean - negative type - with regard to the week variable

negative type = 26,140 + 1,5738 * week

Correlation: r,40336
The above diagrams show that the diurnal variability of the index is very high. The EMN index is extremely sensitive to the influence of various environmental factors, especially to alcohol and stressing situations. It is interesting to observe in what way the EMN values decrease as a result of an illness affecting an individual and then a gradually increase after recuperation and when the general state of the human system improves. (see diagram 3 for the negative type when the value of his EMN index grows suddenly in the period of rest and when his lifestyle is stabilised). In spite of the fact that the 24-hour period values of the index are high, the changes recorded in over two months weekly periods indicate that in this short period of time the behaviour of the system has been fairly stabilised. The fluctuations of the mean values of EMN are not high (Diagrams 5-8). The linear regression (Diagrams 9-12) shows that in the case of the positive individual there are no upward or downward trends in the changes of the EMN index across 2-month period. In the case of the negative individual, on the other hand, an upward trend is observed in the changes of the EMN index value. This is due to a few days of stabilised and hygienic lifestyle.

The measurement error was calculated as a quotient of the sum of squares of arithmetic mean deviations within groups and the sum of squares of deviations from the arithmetic mean between the groups. The error was:

For the positive individual:
- repetitive counting 3.42 %
- sequential counting 12.93 %

For the negative individual:
- repetitive counting 4.18 %
- sequential counting 10.22 %

Drawing conclusions from this method of error calculation one should take into consideration the fact that:
- the error for calculations concerning repetitive counting comprises viability of cells, observer’s error and other random errors
- the error for calculations concerning sequential counting comprises viability of cells, observer’s error, variance of place and other random errors

Thus, a smaller repetitive counting error does not necessarily mean that it better reflects the EMN value. It simply indicates that the variance of place is small and that observer makes a relatively small error because he always counts the same place 3 times. Sequential counting entails measurement error with greater variance of place. As a result, in order to reflect the biological condition of an individual, sequential counting of cells every 100 cells on the entire area of the glass and then calculating the mean value of the count is preferable.

Repetitiveness of measurements was appraised through the calculation of correlation coefficients between all measurements 1, 2 and 3 in both methods of counting. The resulting value was very high. The following table shows the results.
<table>
<thead>
<tr>
<th>Negative type sequential measurement</th>
<th>Positive type sequential measurement</th>
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</thead>
<tbody>
<tr>
<td>corr. coef.(1,2)= 0,8894</td>
<td>corr. coef.(1,2)= 0,84056</td>
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<tr>
<td>corr. coef.(1,3)= 0,89234</td>
<td>corr. coef.(1,3)= 0,82965</td>
</tr>
<tr>
<td>corr. coef.(2,3)= 0,84689</td>
<td>corr. coef.(2,3)= 0,83951</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Negative type repetitive measurement</th>
<th>Positive type repetitive measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>corr. coef.(1,2)= 0,95124</td>
<td>corr. coef.(1,2)= 0,9564</td>
</tr>
<tr>
<td>corr. coef.(1,3)= 0,93528</td>
<td>corr. coef.(1,3)= 0,94804</td>
</tr>
<tr>
<td>corr. coef.(2,3)= 0,95573</td>
<td>corr. coef.(2,3)= 0,97345</td>
</tr>
</tbody>
</table>

References
2. MAKAŁOWSKA I., 1992, Przydatność i zakres stosowalności kryteriów oceny wieku biologicznego w badaniach ontogenetycznych na przykładzie metody EMN i metody analizy morfologicznej – Typescript of doctoral thesis. Adam Mickiewicz University Poznań