OSTEOCALCIN (MARKER FOR THE BONE FORMATION PROCESS OSTEOBLASTIC ACTIVITY) INTRODUCTION OF NEW METHODS INTO ROUTINE PRACTICE, DETERMINE THE REFERENCE RANGE AND STABILITY OF SAMPLES

NEMOCNICE VE FRÝDKU-MÍSTKU

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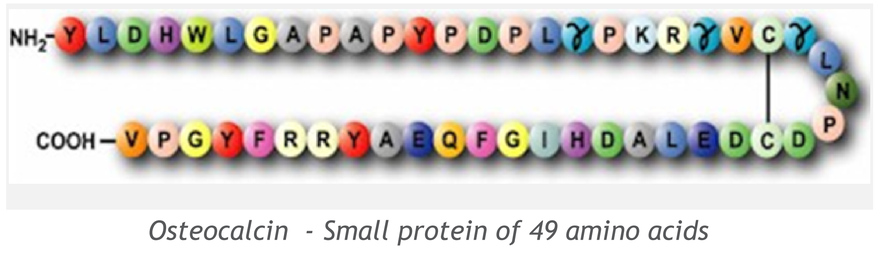
INTRODUCTION

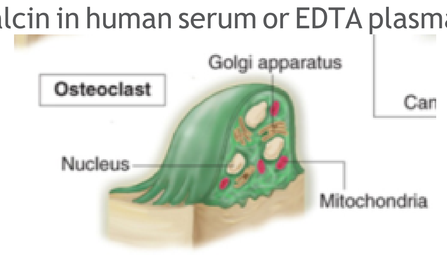
Osteocalcin (OC) is a non-collagenous protein found in the extracellular matrix of bone and dentin. OC involved in regulating mineralization in the bones and teeth. As OC secreted solely by osteoblasts, it is often used as a marker for the bone formation process (osteoblastic activity). Osteocalcin concentration depends on the age and stage of sexual maturation.



Osteocalcin - Small protein of 49 amino acids

AIM OF THE STUDY

Introduction of new methods of osteocalcin in routine practice test using ST AIA-PACK OC TOSOH BIOSCIENCE. Determine the reference range for each age group and stability of Osteocalcin in human serum or EDTA plasma.



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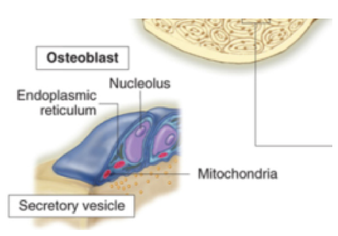

Rebuilding

METHODS AND RESULTS

For the purpose of determining the reference range were analyzed values measured in plasma and serum in three groups of patients. **Group 0** - 80 menopausal patients, **Group 1** - 90 female patients of childbearing age and men - **Group 2** - where 101 patients took part. A group of 271 respondents of various ages was selected as individuals with no health problem.

In both cases (serum, plasma) were analysis of variance (ANOVA) revealed significant differences in the diameters of the individual groups (0,1,2), that is why were the reference limits in both cases set for each group separately.

Analysis of variance assumes independence selections, normality and identical variances. Neither of these assumptions is violated, in both cases (serum and plasma) we have three independent samples that have not rejected the hypothesis of normality of distribution (Kolmogorov -Smirnov test see table 1.) nor the hypothesis of conformity of variance (Leven test see table 2.). Results of ANOVA - see table 3.

Kolmogorov -Smirnov test *see table 1.*

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of serum0 is normal with mean 25.048 and standard deviation 11.639.	One-Sample Kolmogorov-Smirnov Test	.204	Retain the null hypothesis.
2	The distribution of plazma0 is normal with mean 29.046 and standard deviation 13.219.	One-Sample Kolmogorov-Smirnov Test	.058	Retain the null hypothesis.
3	The distribution of serum1 is normal with mean 20.172 and standard deviation 9.497.	One-Sample Kolmogorov-Smirnov Test	.079	Retain the null hypothesis.
4	The distribution of plazma1 is normal with mean 23.628 and standard deviation 11.044.	One-Sample Kolmogorov-Smirnov Test	.103	Retain the null hypothesis.
5	The distribution of serum2 is normal with mean 22.791 and standard deviation 11.484.	One-Sample Kolmogorov-Smirnov Test	.106	Retain the null hypothesis.
6	The distribution of plazma2 is normal with mean 26.66 and standard deviation 12.963.	One-Sample Kolmogorov-Smirnov Test	.231	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Leven test *see table 2.*

Test of Homogeneity of Variances

	Levene Statistic	*df1*	*df2*	*Sig.*
serum	2,137	2	268	,120
plazma	1,195	2	268	,304

ANOVA - *see table 3.*

		Sum of Squares	*Df*	*Mean Square*	*F*	*Sig.*
serum	Between Groups	1013,231	2	506,62	4,25	,015
	Within Groups	31916,416	268	119,09		
	Total	32929,646	270			
plazma	Between Groups	1258,343	2	629,17	4,07	,018
	Within Groups	41461,723	268	154,71		
	Total	42720,066	270			

Determination of reference range for human serum osteocalcin

1) Group 0 - 80 menopausal patients (from the original set of 80 measurements were removed 2 outliers)

Menopausal patients						
Specime	78					
Mean:	24,06					
Std. Deviation:	9,88					
Reference Range:	**4,71 - 43,42**					
Lower Bound	Lower Limit Lower Bound	Upper Limit Lower Bound	Upper Bound	Lower Limit Upper Bound	Upper Limit Upper Bound	Error confidence interval
4,71	1,57	7,84	43,42	40,29	46,56	16%

2) Group 1 - 90 female patients of childbearing age (from the original set of 90 measurements were removed 7 outliers)

Female patients of childbearing age						
Specimens:	83					
Mean:	18,07					
Std. Deviation:	6,29					
Reference Range:	**5,75 - 30,39**					
Lower Bound	Lower Limit Lower Bound	Upper Limit Lower Bound	Upper Bound	Lower Limit Upper Bound	Upper Limit Upper Bound	Error confidence interval
5,75	3,82	7,68	30,39	28,46	32,32	16%

3) Group 2 - men (from the original set of 101 measurements were removed 3 outliers)

Skupina 2 - muži						
Specimens:	98					
Mean:	21,92					
Std. Deviation:	10,44					
Reference Range:	**1,46 - 42,38**					
Lower Bound	Lower Limit Lower Bound	Upper Limit Lower Bound	Upper Bound	Lower Limit Upper Bound	Upper Limit Upper Bound	Error confidence interval
1,46	-1,49	4,42	42,38	39,42	45,33	14%

Determination of reference range for plasma osteocalcin

1) Group 0 - 80 menopausal patients (from the original set of 80 measurements were removed 2 outliers)

Menopausal patients						
Specimens:	78					
Mean:	27,89					
Std. Deviation:	10,93					
Reference Range:	**6,48 - 49,31**					
Lower Bound	Lower Limit Lower Bound	Upper Limit Lower Bound	Upper Bound	Lower Limit Upper Bound	Upper Limit Upper Bound	Error confidence interval
6,48	3,01	9,95	49,31	45,84	52,77	16%

2) Group 1 - 90 female patients of childbearing age (from the original set of 90 measurements were removed 7 outliers)

Female patients of childbearing age						
Specimens:	83					
Mean:	21,21					
Std. Deviation:	7,45					
Reference Range:	**6,61 - 35,82**					
Lower Bound	Lower Limit Lower Bound	Upper Limit Lower Bound	Upper Bound	Lower Limit Upper Bound	Upper Limit Upper Bound	Error confidence interval
6,61	4,31	8,9	35,82	33,53	38,11	16%

3) Group 2 - men (from the original set of 101 measurements were removed 3 outliers)

Men						
Specimens:	99					
Mean:	25,92					
Std. Deviation:	11,94					
Reference Range:	**2,52 - 49,33**					
Lower Bound	Lower Limit Lower Bound	Upper Limit Lower Bound	Upper Bound	Lower Limit Upper Bound	Upper Limit Upper Bound	Error confidence interval
2,52	-0,85	5,88	49,33	45,96	52,69	14%

Reference ranges and their confidence intervals were determined by the following formulas (viz [1]):

Lower Bound: $x_D = \bar{x} - 1{,}96\,s$ Upper Bound: $x_H = \bar{x} + 1{,}96\,s$

Lower Limit Lower Bound : $x_{DD} = \bar{x} - 1{,}96\,s - 1{,}64\sqrt{s^2/n + 1{,}96^2 s^2/(2n)}$

Upper Limit Lower Bound: $x_{HD} = \bar{x} - 1{,}96\,s + 1{,}64\sqrt{s^2/n + 1{,}96^2 s^2/(2n)}$

Lower Limit Upper Bound: $x_{DH} = \bar{x} + 1{,}96\,s - 1{,}64\sqrt{s^2/n + 1{,}96^2 s^2/(2n)}$

Upper Limit Upper Bound: $x_{HH} = \bar{x} + 1{,}96\,s + 1{,}64\sqrt{s^2/n + 1{,}96^2 s^2/(2n)}$

**Mean, ˢSelective SD, ⁿQuantity of measurements*

Results of osteocalcin in human serum and in EDTA plasma

Furthermore, we compared the results of measurements obtained from serum and from plasma in each of the 271 respondents. The aim of analysis was to determine whether these two procedures give the same results. Based on a comparison of mean values is seen that the results of analysis of serum and plasma differ (see table 4.).

see table 4.

Paired Samples Statistics

		Mean	*N*	*Std. Deviation*	*Std. Error Mean*
Pair 1	Serum	22,59	271	11,04362	,67085
	Plazma	26,36	271	12,57865	,7641

Whether this difference is statistically significant , was tested by Wilcoxon and sign test for two dependent samples. Both tests consistently rejected the hypothesis of a zero median difference of paired values, which clearly demonstrated the differences between the two techniques. The values obtained by the analysis of serum osteocalcin differ significantly from the values obtained by analyzing the plasma (see

see table 5.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The median of differences between plazma and serum equals 0.	Related-Samples Sign Test	.000	Reject the null hypothesis.
2	The median of differences between serum and plazma equals 0.	Related-Samples Wilcoxon Signed Ranks Test	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Results of osteocalcin before and after freezing

Comparison of measurement results obtained before and after freezing, we had 20 samples, where were the values of osteocalcin measured before and then six weeks after freezing (see table 6.).

see table 6.

Paired Samples Statistics

		Mean	*N*	*Std. Deviation*	*Std. Error Mean*
Pair 1	before	28,89	20	14,172	3,169
	after	25,17	20	13,784	3,082

For comparison, the values obtained before and after freezing was used paired t-test (see table 7.).

see table 7.

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 before - after	3,720	2,276	,509	2,655	4,785	7,308	19	,000

This test confirmed that osteocalcin values obtained before and 6 weeks after freezing differ significantly. The difference between these values was found to be statistically significant at the 1% significance level (p- value = 0.000). Osteocalcin values ??obtained 6 weeks after freezing are in the average 3.72 smaller than the values obtained before freezing (see table 8).

see table 8.

Descriptives

			Statistic	Std. Error
before	Mean		3,7200	,50904
min	95% Confidence Interval for	Lower Bound	2,6546	
after	Mean	Upper Bound	4,7854	
	5% Trimmed Mean		3,6122	
	Median		3,3700	
	Variance		5,182	
	Std. Deviation		2,27649	
	Minimum		,25	
	Maximum		9,13	
	Range		8,88	
	Interquartile Range		3,1	
	Skewness		,56	,512
	Kurtosis		,284	,992

CONSLUSIONS

Determination of reference ranges for human serum osteocalcin	Group 0	Group 1	Group 2
Mean (ng/ml)	24.6	18.7	21.92
Reference Range (ng/ml)	4.71 - 43.42	5.75 - 30.39	1.46 - 42.38

Determination of reference ranges for plasma osteocalcin	Group 0	Group 1	Group 2
Mean (ng/ml)	27.89	21.21	25.92
Reference Range (ng/ml)	6.48 - 49.31	6.61 - 35.82	2.52 - 49.33

Literatura
[1] Jabor, A., Franeková, J. Principy interpretace laboratorních testů. Praha: Roche, s.r.o., Diagnostic Division, 2013. ISBN 978-80-260-5094-0.
[2] Lothar Thomas. Clinical Laboratory Diagnostics. TH Books Verlagsgesellschaft mbH, Frankfurt/Main,Germany, 1998. ISBN 3-9805215-4-0.

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