Original Article

건강검진이 의뢰된 여성의 연령에 따른 Osteocalcin의 참고범위 설정

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Establishment of Reference Intervals of Osteocalcin according to Age in Women for Health Promotion Center

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Purpose: Osteocalcin is also known as the bone gamma-carboxyglutamic acid (Gla) protein (BGP), is non-collagenous bone protein synthesized by osteoblasts. Serum concentrations of Osteocalcin have been used as a biochemical marker of bone turnover. The reference intervals of Osteocalcin is categorized by kit corporation according to the age. However, each laboratory should establish its own reference intervals. In this study, the variation in the serum Osteocalcin level were used to find actual standard age-specific Osteocalcin reference intervals. **Materials and Methods:** We have selected 864 healthy females aged 20~80 years who visited a health promotion center between Aug. 2007 and Sep. 2008. The Osteocalcin IRMA Kit (OSTEO-RIACT, CIS Bio international, Gif-sur-Yvette, France) was used for the quantification. Each results were analyzed with the SPSS 12.0 statistical software. **Results:** The analyzed reference intervals of Osteocalcin by using Hoffmann method are from 8.8~39.4 ng/mL to 6.3~28.8 ng/mL for the case of the age from 20 to 30, from 7.7~31.9 ng/mL to 5.9~17.4 ng/mL for the case of the age from 31 to 40, and from 8.0~36.0 ng/mL to 5.5~20.1 ng/mL for the case of the age from 41 to 50, and from 8.0~50.5 ng/mL to 6.7~27.0 ng/mL for the case of the age from 51 to 60, and from 12.9~55.9 ng/mL to 7.5~27.5 ng/mL for the case of the age from 61 to 80. Reference intervals of Osteocalcin were not in agreement with those recommended by the manufacturers. **Conclusions:** Osteocalcin is used as an indication of metabolic bone diseases. So in our study we wanted to provide reference intervals of Osteocalcin that can be useful to a clinical decisions. Also, previous reference intervals should not be re-used and new intervals should be set by continuous analyzing. **(Korean J Nucl Med Technol 2009;13(1):104-111)**

Key Words : Osteocalcin, Reference intervals, Hoffmann method

INTRODUCTION

Osteocalcin is also known as bone gamma-carboxy glutamic acid (Gla) protein (BGP), is non-collagenous bone protein synthesized by osteoblasts.[1-3] Serum concentrations of Osteocalcin have been used as a biochemical marker of bone turnover. Serum Osteo calcin is elevated in disease states characterized by increased bone turnover such as hyperparathyroidism, Paget's disease and low in conditions associated with low bone turnover such as hypoparathyroidism and growth hormone deficiency. It has also been useful in the research of toxic effects on bone. The reference intervals of Osteocalcin is categorized by KIT corporation according to the age. However, each laboratory should establish its own reference intervals.[4] In this study, the variation in the serum Osteocalcin level were used to find actual standard age-specific Osteocalcin reference intervals. The serum concentration of Osteocalcin is measured by immunoradiometric assay

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(IRMA).[5] And by using Hoffmann method established age-specific Osteocalcin reference intervals.

MATERIALS AND METHODS

1. Materials

We have selected 864 normal healthy female adults who took health promotion center between Aug. 2007 and Sep. 2008. We studied within the age range of 20~80 years. We classified normal healthy female adults into five age groups (Table 1).

Table 1. Age distribution of subjects

Group	Age ranges	Female
A	20~30	50
B	31~40	127
C	41~50	317
D	51~60	272
E	61~80	98
Total		864

Haemolytic sample may yield false-negative low Osteocalcin values. So haemolysed sample was excepted.

2. Specimen Handling

The blood sample should be collected between 8:00 a.m. and 10:00 a.m. The patient should fast over 8 hours. Osteocalcin is subject to a circadian rhythm, in which maximum values are observed at morning and minimum values in the afternoon and early evening. Osteocalcin is markedly unstable in vitro,[6,7] rapidly degrading in samples at room temperature and at 4℃.[8,9] So blood samples should be processed within 2~3 hr after collection and cold-centrifuged if possible. The Osteocalcin IRMA Kit (OSTEO-RIACT, CIS Bio international, Gif-sur-Yvette, France) was used for the quantification. The Principle is seurm contained Osteocalcin is bound by both the immobilized antibody and the labeled antibody to form a sandwich complex.

3. Establishment of reference intervals

1) Statistics analysis: SPSS 12.0 for Windows

2) Establishment of reference intervals: Hoffmann method

3) Observation

① Statistics comparison - Mean value, Deviation, Skewness, Kurtosis

② Frequency distribution table Comparison
- Frequency, %, Accumulation%

③ Comparison of histogram and normal distribution curve-distribution confirmation

4) 2 step analysis

< Step 1>

① The data from the analysis was inputted into the computer.

② Determined the mean value and the standard deviation.

③ Outliers which were over ±3SD from the mean were excluded.

< Step 2>

The method of defining reference intervals

Method A. Hoffmann method

① Determined the mean value and the standard deviation.

② Reference intervals were established by the Mean±2SD.

Method B. Bayesian method

① Determined the mean value and the standard deviation.

② Placed the values in order according to size.

③ Evaluated the confidence intervals

Confidence intervals (CI) were evaluated by using 2.5 percentile (lower reference limit) and 97.5 percentile (upper reference limit).

5) Used analysis statistics:

① Skewness, Kurtosis

Skewness is a measure of the uneveness of the distribution of the data. A positive skewness value (Skewness>0.5) has a long right-hand tail. While a negative skewness value (Skewness<-0.5) has a long left-hand tail. Kurtosis is measure of the peakedness of the bell curve. A positive number indicates more of a peak than standard; a negative number indicates flatness of the line.

② Analysis the distribution of histogram

We transformed the data by taking the natural logarithm of each value. The distribution of the logarithmic data is much closer to the normal distribution. Setting the exponential would raise the value. where e is an irrational constant approximately equal to 2.718281828459.

4. Analysis of Osteocalcin data distribution (Hoffmann method)

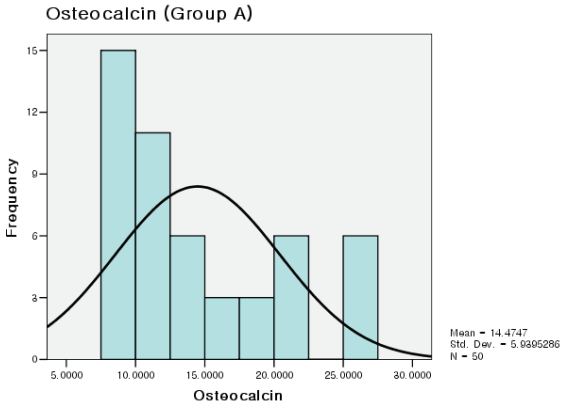

Fig. 1-1. The before conversion (Statistics ; Group A 50 case).

Statistics (Group A)

		Osteocalcin	LnOsteocalcin
N	Valid	50	50
	Missing	0	0
Mean		14.474700	2.597501
Std. Error of Mean		.8399762	.0540229
Median		11.280000	2.423031
Std. Deviation		5.9395286	.3819996
Variance		35.278	.146
Skewness		.880	.517
Std. Error of Skewness		.337	.337
Kurtosis		-.587	-1.089
Std. Error of Kurtosis		.662	.662
Range		18.9590	1.2237
Minimum		7.9000	2.0669
Maximum		26.8590	3.2906
Sum		723.7350	129.8750
Percentiles	2.5	7.900000	2.066863
	25	9.844250	2.286878
	50	11.280000	2.423031
	75	18.755500	2.928535
	97.5	26.859000	3.290601

Fig. 1-2. Comparison between the before and the after conversion (Statistics; Group A 50 case).

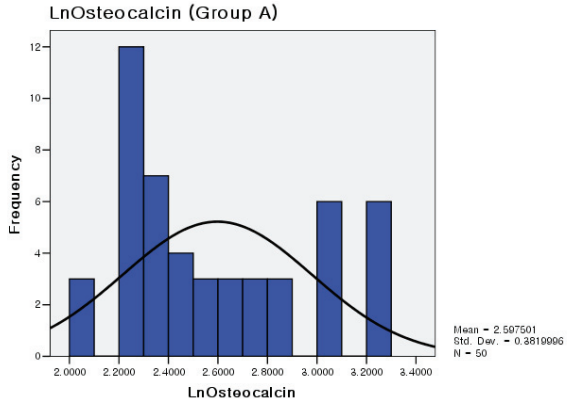

Fig. 1-3. The after conversion (Statistics ; Group A 50 case).

For statistical analysis, distribution of histogram which has been created from test results of different ages has been checked.

1) Group A

The before logarithm conversion the test results were distributed one-sided (Mean=14.4747, SD=5.9395). The value of skewness is positive value 0.8, it's value out of -0.5~0.5. A histogram with a long right-hand tail is said to be skewed to the right (Fig. 1-1, 2). So we concluded that the data do not come from a normal population. We transformed the data, by taking the natural logarithm of each value. The distribution of the logged data is much closer to normal (Mean=2.5975, SD=0.3819). The value of skewness is 0.5, within -0.5~0.5 (Fig. 1-2, 3). To change it to logarithm normal distribution and establish the reference intervals, the average and standard deviation were obtained. The average of LnOsteocalcin is 1.834~3.362. After setting exponential to change moderated values, the reference intervals are 6.3~28.8 ng/mL and the average is 13.4 ng/mL.

2) Group B

The before logarithm conversion the test results were distributed one-sided (Mean=10.5100, SD=2.9944). The value of skewness is positive value 1.0, it's value out of -0.5~0.5. A histogram with a long right-hand tail is said to be skewed to the right (Fig. 2-1, 2). So we concluded that the data do not come from a normal population. We transformed the data, by taking the natural logarithm of each value. The distribution of the logged data is much closer to normal (Mean=2.3152, SD=0.2701). The value of skewness is 0.2, within -0.5~0.5 (Fig. 2-2, 3). To change it to logarithm normal distribution and establish the reference intervals, the average and standard deviation were

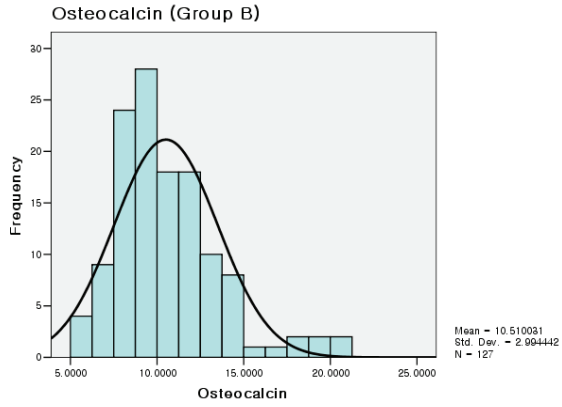

Fig. 2-1. Before the conversion (Statistics; Group B 127 case).

Statistics (Group B)

		Osteocalcin	LnOsteocalcin
N	Valid	127	127
	Missing	0	0
Mean		10.510031	2.315229
Std. Error of Mean		.2657138	.0239684
Median		9.900000	2.292535
Std. Deviation		2.9944420	.2701107
Variance		8.967	.073
Skewness		1.068	.291
Std. Error of Skewness		.215	.215
Kurtosis		1.308	-.026
Std. Error of Kurtosis		.427	.427
Range		14.3480	1.2601
Minimum		5.6810	1.7371
Maximum		20.0290	2.9972
Sum		1334.7740	294.0341
Percentiles	2.5	6.009000	1.793254
	25	8.271000	2.112755
	50	9.900000	2.292535
	75	11.950000	2.480731
	97.5	18.800000	2.933857

Fig. 2-2. Comparison between the before and the after conversion (Statistics; Group B 127 case).

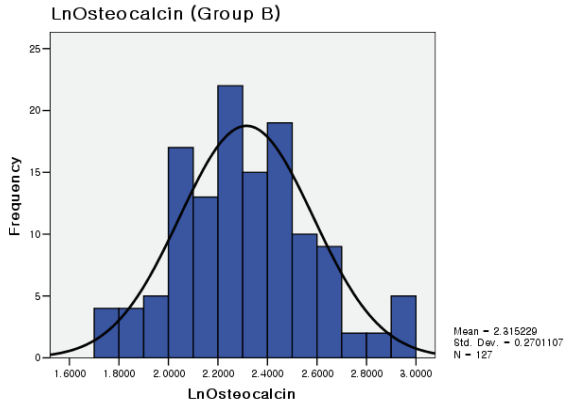

Fig. 2-3. The after conversion (Statistics; Group B 127 case).

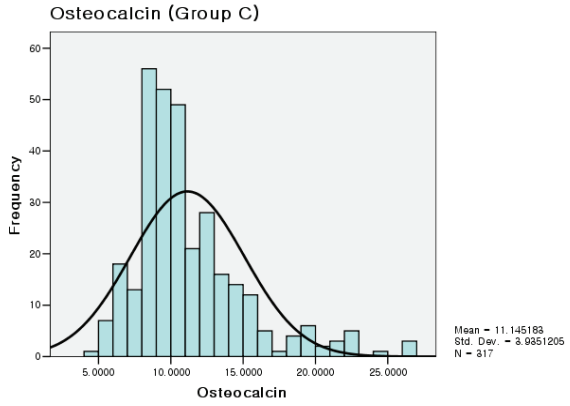

Fig. 3-1. The before conversion (Statistics; Group C 317 case).

obtained. The average of LnOsteocalcin is 1.775~2.855. After setting exponential to change moderated values, the reference intervals are 5.9~17.4 ng/mL and the average is 10.1 ng/mL.

3) Group C

The before logarithm conversion the test results were distributed one-sided (Mean=11.1451, SD=3.9351). The value of skewness is positive value 1.4, it's value out of -0.5~0.5. A histogram with a long right-hand tail is said to be skewed to the right (Fig. 3-1, 2). So we concluded that the data do not come from a normal population. We transformed the data, by taking the natural logarithm of each value. The distribution of the

Statistics (Group C)

		Osteocalcin	LnOsteocalcin
N	Valid	317	317
	Missing	0	0
Mean		11.145183	2.356925
Std. Error of Mean		.2210184	.0181170
Median		10.293000	2.331500
Std. Deviation		3.9351205	.3225636
Variance		15.485	.104
Skewness		1.437	.381
Std. Error of Skewness		.137	.137
Kurtosis		2.524	.360
Std. Error of Kurtosis		.273	.273
Range		22.0800	1.7910
Minimum		4.4200	1.4861
Maximum		26.5000	3.2771
Sum		3533.0230	747.1452
Percentiles	2.5	5.670750	1.735230
	25	8.607500	2.152650
	50	10.293000	2.331500
	75	12.699000	2.541500
	97.5	22.277700	3.103620

Fig. 3-2. Comparison between the before and the after conversion (Statistics; Group C 317 case)

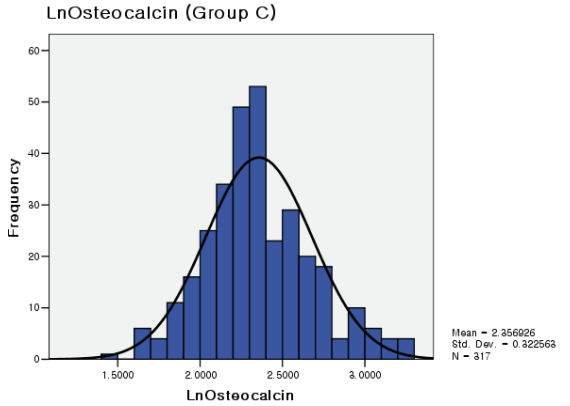

Fig. 3-3. The after conversion (Statistics; Group C 317 case).

logged data is much closer to normal (Mean=2.3569, SD=0.3225). The value of skewness is 0.3, within -0.5~0.5 (Fig. 3-2, 3). To change it to logarithm normal distribution and establish the reference intervals, the average and standard devation were obtained. The average of LnOsteocalcin is 1.712~3.002. After setting exponential to change moderated values, the reference intervals are 5.5~20.1 ng/mL and the average is 10.6 ng/mL.

4) Group D

The before logarithm conversion the test results were distributed one-sided (Mean=14.278, SD=5.115). The value of skewness is positive value 1.18, it's value out of -0.5~0.5. A histogram with a long right-hand tail is said to be skewed to the right (Fig. 4-1, 2). So we concluded that the data do not come from a normal population. We transformed the data, by taking the natural logarithm of each value. The distribution of the logged data is much closer to normal (Mean=2.598, SD=0.347). The value of skewness is -0.04, within -0.5~0.5 (Fig. 4-2, 3). To change it to logarithm normal distribution and establish the reference intervals, the average and standard deviation were obtained. The average of LnOsteocalcin is 1.903~3.294. After setting exponential to change moderated values, the reference intervals are 6.7~27.0 ng/mL and the average is 13.4 ng/mL.

5) Group E

Before logarithm conversion the test results were distributed one-sided (Mean=15.093, SD=4.788). The value of skewness is positive value 0.7, it's value out of -0.5~0.5. A histogram with a long right-hand tail is said to be skewed to the right (Fig. 5-1, 2). So we concluded that the data do not come from a normal population. We transformed the data, by taking the natural logarithm of each value. The distribution of the logged data is much closer to normal (Mean=2.663, SD=0.325). The value of skewness is -0.4, within -0.5~0.5 (Fig. 5-2, 3). To change it to logarithm normal distribution and establish the reference intervals, the average and standard deviation were obtained. The average of LnOsteocalcin is 2.013~3.314. After setting exponential to change moderated values, the reference intervals are 7.5~27.5 ng/mL and the average is 14.4 ng/mL.

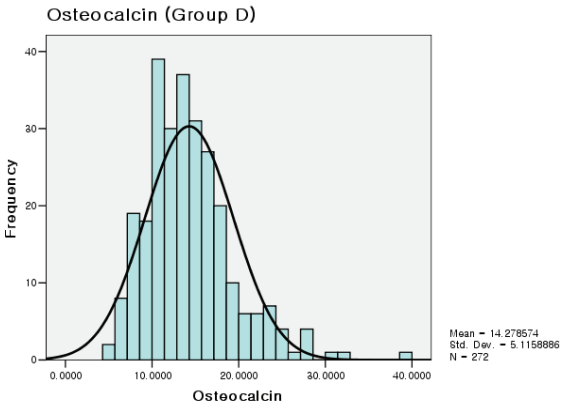

Fig. 4-1. The before conversion (Statistics; Group D 272 case).

Statistics (Group D)

		Osteocalcin	LnOsteocalcin
N	Valid	272	272
	Missing	0	0
Mean		14.278574	2.598687
Std. Error of Mean		.3101963	.0210896
Median		13.599000	2.609996
Std. Deviation		5.1158886	.3478189
Variance		26.172	.121
Skewness		1.184	-.045
Std. Error of Skewness		.148	.148
Kurtosis		2.630	.212
Std. Error of Kurtosis		.294	.294
Range		34.9260	2.0753
Minimum		5.0130	1.6120
Maximum		39.9390	3.6874
Sum		3883.7720	706.8428
Percentiles	2.5	6.444550	1.863230
	25	10.700000	2.370244
	50	13.599000	2.609996
	75	16.744000	2.818040
	97.5	27.281550	3.306196

Fig. 4-2. Comparison between the before and the after conversion (Statistics; Group D 272 case).

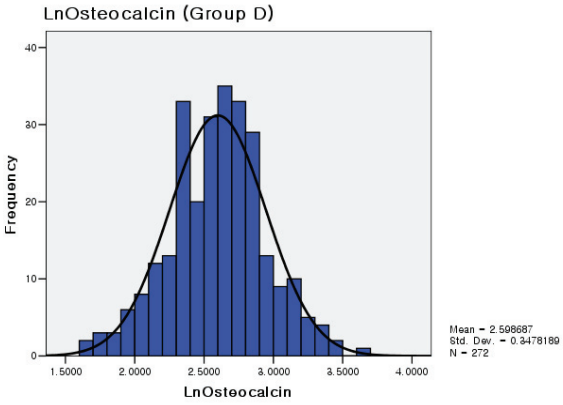

Fig. 4-3. The after conversion (Statistics; Group D 272 case).

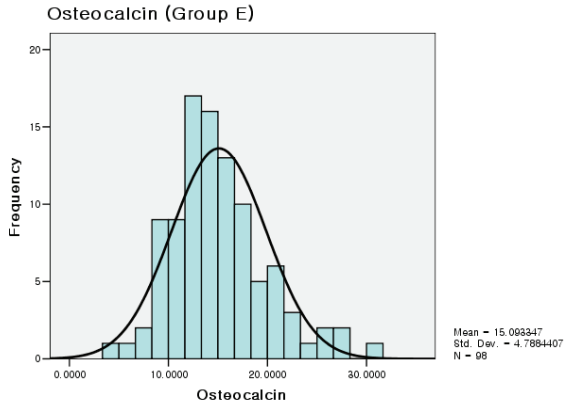

Fig. 5-1. The before conversion (Statistics; Group E 98 case).

Statistics (Group E)

		Osteocalcin	LnOsteocalcin
N	Valid	98	98
	Missing	0	0
Mean		15.093347	2.663979
Std. Error of Mean		.4837056	.0328552
Median		14.743500	2.690798
Std. Deviation		4.7884407	.3252500
Variance		22.929	.106
Skewness		.753	-.465
Std. Error of Skewness		.244	.244
Kurtosis		.854	1.445
Std. Error of Kurtosis		.483	.483
Range		26.1290	1.9770
Minimum		4.2000	1.4351
Maximum		30.3290	3.4121
Sum		1479.1480	261.0700
Percentiles	2.5	7.107125	1.952233
	25	11.869750	2.473987
	50	14.743500	2.690798
	75	17.646500	2.870535
	97.5	27.514750	3.314697

Fig. 5-2. Comparison between the before and the after conversion (Statistics; Group E 98 case).

6) Result of Comparison between Methods in Defining Reference Intervals at a specific age groups.

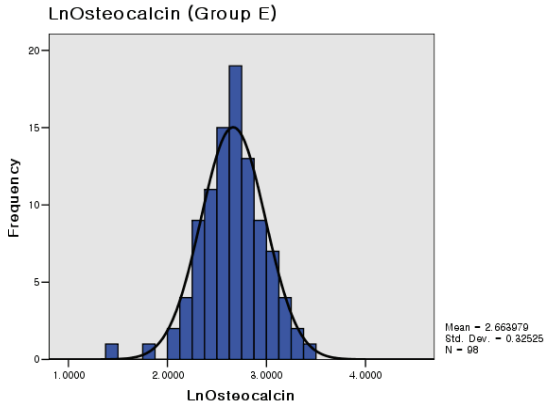

Fig. 5-3. The after conversion (Statistics; Group E 98 case).

Before the conversion, you can see that there is quite a difference between the two reference intervals (Table 2), one determined by the Hoffmann method and the other determined by the Bayesian method. On the other hand, after the conversion, there is only a little difference between the two reference intervals (Table 2). Therefore the distribution without a definite normal distribution should be converted to have a normal distribution. With Hoffmann method, we can analyze the data distribution rather precisely by checking the data per each intervals. And with Bayesian method, we can analyze the overall distribution based on quartiles on the box plot.

RESULTS

The average range was obtained through the Hoffmann method that acquires reference intervals within 95% confidence intervals. According to the age groups through the Hoffmann method that acquires the results are as follows:

1) In case of Group A

The average of LnOsteocalcin is 1.834~3.362. After setting exponential to change moderated values, the reference intervals are 6.3~28.8 ng/mL and the average is 13.4 ng/mL. Recommended by

Table 2. Comparison of establishment method for reference intervals

Age (yr)	The before conversion		The after conversion	
	Hoffmann	Bayesian	Hoffmann	Bayesian
20~30	2.596~26.354	7.9~26.8	6.256~28.832	7.9~26.9
31~40	4.521~16.491	6~18.8	5.900~17.382	6.045~18.8
41~50	3.275~19.015	5.685~22.5	5.539~20.127	5.685~22.5
51~60	4.047~24.510	6.4~27.6	6.706~26.959	6.4~27.6
61~80	5.516~24.670	6.207~27.7	7.489~27.508	6.207~27.7

the manufacturers' reference intervals are 8.8~39.4 ng/mL.

2) In case of Group B

The average of LnOsteocalcin is 1.775~2.855. After setting exponential to change moderated values, the reference intervals are 5.9~17.4 ng/mL and the average is 10.1 ng/mL. Recommended by the manufacturers' reference intervals are 7.7~31.9 ng/mL.

3) In case of Group C

The average of LnOsteocalcin is 1.712~3.002. After setting exponential to change moderated values, the reference intervals are 5.5~20.1 ng/mL and the average is 10.6 ng/mL. Recommended by the manufacturers' reference intervals are 8.0~36.0 ng/mL.

4) In case of Group D

The average of LnOsteocalcin is 1.903~3.294. After setting exponential to change moderated values, the reference intervals are 6.7~27.0 ng/mL and the average is 13.4 ng/mL. Recommended by the manufacturers' reference intervals are 8.0~50.5 ng/mL.

5) In case of Group E

The average of LnOsteocalcin is 2.013~3.314. After setting exponential to change moderated values, the reference intervals are 7.5~27.5 ng/mL and the average is 14.4 ng/mL. Recommended by the manufacturers' reference intervals are 12.9~55.9 ng/mL. Reference intervals of Osteocalcin were not in agreement with those recommended by the manufacturers.

CONCLUSION

Osteocalcin is used as an indication of metabolic bone diseases. So in our study we wanted to provide reference intervals of Osteocalcin that can be useful to a clinical decisions. Osteocalcin is used as a non-invasive marker of bone turnover.

Factor such as diet, age, sex, and renal function affect the results of biochemic marker and should be appropriately adusted whenever possible. In accordance with regard to the influential medical treatment prior to the experiment, there perhaps exist fluctuations. Consequently, the experiment requires being in accordance with identical parameters.

In this study, there are insufficient numbers of participants who are in the range of 20~30 years and a similar inadequacy occurs with those over 80 years of age who have visited a health promotion center. As a result the sample population has defined set limits to those participants falling within the age range 20~80 years old age groups. Nevertheless, there remains the fact that there is an increasing elderly population. Thus what has been the cut off point of 80 years at the present time can no longer be ignored, indication a requirement to include this age groups.

Also, previous reference intervals should not be re-used and new intervals should be set by continuous analyzing.

요 약

목적 : Bone Gla protein (BGP)라고도 불리는 Osteocalcin은 조골세포에 의해 합성되는 비 콜라겐성 뼈 단백질이다. 혈청 Osteocalcin은 골 대사의 생화학적 지표로 이용되고 있다. Osteocalcin수치의 참고치는 연령에 따라 제조회사에서 권장하는 참고치가 있으나 검사실 자체적으로 정하도록 권고하고 있다. 본 연구는 현재 사용되어지고 있는 연령별 Osteocalcin 참고치를 재설정해 보고하고자 하였다.

실험재료 및 방법 : 2007년 8월부터 2008년 9월 사이에 본원 건강검진 센터를 방문하였던 20~80세의 정상 성인 여자 864명을 대상으로 하였다. 혈청 Osteocalcin은 면역방사계측법(IRMA)을 이용한 Kit (OSTEO-RIACT, CIS Bio international, Gif-sur-Yvette, France)를 사용하였으며, 각각의 결과는 통계 프로그램인 SPSS 12.0을 이용하여 분석하였다.

결과 : Hoffmann 방법을 시행하여 얻은 Osteocalcin 참고치는 다음과 같다. 20~30세에서는 8.8~39.4 ng/mL에서 6.3~28.8 ng/mL로 31~40세에서는 7.7~31.9 ng/mL에서 5.9~17.4 ng/mL로 41~50세에서는 8.0~36.0 ng/mL에서 5.5~20.1 ng/mL로 51~60세에서는 8.0~50.5 ng/mL에서 6.7~27.0 ng/mL로 61~80세에서는 12.9~55.9 ng/mL에서 7.5~27.5 ng/mL로 변경되었다. Osteocalcin 참고치는 제조회사 권장 참고치와 차이가 있었다.

결론 : 대사성 골질환의 지료로서 사용되어지고 있는 Osteocalcin의 참고치를 정확하게 제공함으로서 임상적 의사결정에 유용하게 이용할 수 있다. 한 번 정한 참고치는 계속 사용하지 말고 지속적으로 재평가를 시행해야 할 것으로 사료된다.

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