Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/7584
DOI URL: http://dx.doi.org/10.21474/IJAR01/7584

RESEARCH ARTICLE

REFERENCE INTERVALS OF SERUM HYALURONIC ACID IN AN ADULT NIGERIAN POPULATION: A PILOT STUDY.

Chima Collins Ihegihu[1], Ebere Yvonne Ihegihu[2], Emmanuel Chiebuka Okoye[3], Kenneth Umezulike Ani[4] and Chika Echezona Anumba[5].

1. Department of Orthopaedic Surgery, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.
2. Department of Physiotherapy, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.
3. Department of Medical Rehabilitation, Faculty of Health Sciences, Nnamdi Azikiwe University Nnewi, Anambra State, Nigeria.
4. Department of Medical Laboratory Services, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

Manuscript Info

Manuscript History

Received: 17 June 2018
Final Accepted: 19 July 2018
Published: August 2018

Keywords:-
Reference intervals, Hyaluronic acid, Knee osteoarthritis, Pilot study, Nigeria

Abstract

Purpose: To determine age and gender-specific reference intervals of hyaluronic acid (HA) in healthy Nigerian Igbos living at Nnewi, as reference for interpreting clinical laboratory values of HA.

Materials And Methods: Eighty healthy volunteers were partitioned into two age groups: group 1 (participants 30 years and above) and group 2 (participants 29 years and below). Each group comprised twenty males and twenty females. Five milliliters of venous blood was collected from each participant and measured for serum level of HA by using the CORGENIX HA Test kit (Broomfield, Colorado, USA). Obtained data was analysed using descriptive statistics of frequency count, range, mean and standard deviation, and inferential statistics of independent t-test and Pearson product moment correlation at 0.05 level of significance.

Results: There were significant correlations between serum HA and each of age ($r=0.76; p<0.01$) and BMI ($r=-0.34; p=0.03$) among the participants. The males had significantly higher level of serum HA than their female counterparts in each group ($p<0.05$). Group1 participants had significantly higher HA level than group 2 participants ($t=9.49; p<0.01$). In group 1, the reference interval for serum HA level for males and females were 19.44 - 25.87ng/ml and 19.55 - 23.35ng/ml respectively whereas the reference interval for the males and females in group 2 were 15.12 - 23.10ng/ml and 13.42 - 21.96ng/ml respectively.

Conclusion: Reference levels of serum HA increase with age and tend to be higher in males than females. Multi-center studies using larger sample sizes are recommended to establish reference intervals for the Nigerian population.

Corresponding Author:- Chima Collins Ihegihu.
Address:- Department of Orthopaedic Surgery, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

Introduction:-

Hyaluronic acid (HA) also known as hyaluronan is an anionic, nonsulphated high molecular weight glycosaminoglycan; one of the chief components of the extracellular matrix (ECM) (Fraser et al, 1997). It is composed of alternating subunits of glucosamine and glucuronic acid and is synthesized by fibroblasts and cells of the ECM (Gamero et al, 2000). Hyaluronic acid is a major and important component of articular cartilage and synovial fluid. It increases the viscosity of the synovial fluid and along with lubricin is one of the fluid's main lubricating components. It also contributes to the resistance to compression of articular cartilage and plays a role in structural properties as well as in cell signaling (Holmes et al, 1988). Hyaluronic acid is a biomarker of osteoarthritis (OA) and an indicator of normal biologic and pathogenic processes in the knee (Biomarkers Definitions Working Groups, 2001; Frank and Hargreves, 2003). It is released into biological fluids and the systemic circulation during bone turnover (Rousseau and Pierre, 2007). Levels of serum HA can differentiate between joints affected by OA and non-affected joints, and also reflects degrees of degradation in the articular cartilage (Kane, 2007).

Osteoarthritis is a disease process which not only affects the cartilage but involves the entire joint, including the subchondral bone, ligaments, synovial membrane, capsule, and the peri-articular muscles leading to cartilage degeneration with fibrillation, fissures, ulcerations and full thickness loss of the joint surface. The disease process can begin as early as the age of 30 years. However, it usually takes years to manifest clinically and appear radiologically. Knee osteoarthritis (KOA) progresses over a long period that includes early molecular and pre-radiographic stages before the late radiographic stage. Knee osteoarthritis is traditionally diagnosed on the basis of clinical and radiological findings supported by laboratory tests such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Though CRP and ESR are indicators of inflammation and may be raised in osteoarthritis, they are not specific indicators of osteoarthritis. However, radiographic KOA indicates the stable structural condition of this disease rather than the activity of the disease at the time of diagnosis (Turan et al, 2007). Usually, joint tissue degeneration is already advanced by the time radiographs provide positive results (Gamero et al, 2000). Whilst arthroscopy, magnetic resonance imaging and ultrasonography are used to assess cartilage lesions and can diagnose early KOA, cost and availability prevents their routine use (Davies-Tuck et al, 2008; Kamei et al, 2008; Lee et al, 2008).

The need for less invasive and cheaper methods of diagnosing and assessing early disease activity has led to the study of biomarkers for cartilage and bone turnover as well as synovitis such as HA (Wollheim, 1999). It has been reported in a previous study that increased levels of serum HA was associated with the presence of radiographic KOA and that changes in levels of serum HA can be seen earlier than radiological changes in patients with KOA (Elliott et al, 2005). It was also reported in a previous study that in patients with KOA, the serum HA level correlated with the degree of synovial proliferation, the length of osteophytes and the degree of joint space narrowing (Turan et al, 2007). Other researchers have also reported that increase in serum level of HA was as a result of synovial inflammation and cartilage degradation which occurred in the disease condition. Thus, measurement of the HA level in serum is useful in assessing the activity as well as predicting development and progression of KOA (Sharif et al, 1995; Pavelka et al, 2004). Early diagnosis and prediction of progression are of particular importance from the standpoint of prevention and therapeutic intervention but so far to the knowledge of the researchers, HA has not entered routine clinical use as a marker for the diagnosis of pre-radiographic knee OA. The objective of this study was to determine the reference intervals of HA for healthy, asymptomatic Nigerians at Nnewi; a town in the south-eastern part of Nigeria inhabited by individuals of Igbo ethnicity. Comparing laboratory values of serum HA of patients who have knee symptoms without radiological features with the gender and age specific reference interval may enable physicians make diagnosis of early KOA.

Materials And Methods:-

Sample population:-

Eighty healthy volunteers with no history or clinical features suggestive of OA of the knee and with absent of radiographic features of OA (Kellgren and Lawrence grade 0 (k/L 0) (Kellgren and Lawrence, 1963) were recruited into the study. Ethical approval was obtained from the Hospital Ethical Committee and written and signed informed consent was given by all the subjects that participated in the study. The participants comprised of forty males and forty females who were all Igbos. They were partitioned into two age groups: group 1 (those 30 years and above) and group 2 (those 29 years and below). There were twenty males and twenty females in each group. Clinical history, physical examination findings and anthropometric measurements of weight and height were documented. The participants' age (in years), height (in metres) and weight (in kilograms) were recorded while body mass index

(BMI) was calculated in kilogram/metres^2 (kg/m^2). Bilateral anterior-posterior and lateral weight-bearing radiographs of the knees were also obtained from each participant on the same day the blood samples were collected. The radiographs were reported by a single radiologist using the K/L atlas for overall radiographic grading. Kellgren and Lawrence grade 0 was defined as showing no radiographic features of OA.

Selection criteria:-
Volunteers were excluded if they had any clinical or radiological features of osteoarthritis or rheumatoid arthritis affecting any joints in the body including the hand joints, shoulders, hips or spine. Volunteers were excluded if they were suffering from renal, hepatic or malignant disease, progressive systemic sclerosis (PSS), systemic lupus erythematosus (SLE); if they had abused alcohol or drugs; if they were pregnant or lactating; or if they were taking oral non-steroidal anti-inflammatory drugs (NSAIDs). Only healthy individuals whose radiographs did not show features of osteoarthritis (K/L 0) or any knee pathology were included in the study.

HA analyses:-
Five milliliters of venous blood sample was collected from the vena mediana cubitii of each participant into an EDTA glass tube taking all aseptic precautions by a board certified laboratory scientist. Serum was separated from cells by centrifugation and stored at 10^{o}C until all the samples were obtained. The samples were then frozen at -20°C and stored until analysis. The blood samples were measured for serum levels of HA by using the CORGENIX HA Test kit (Broomfield, Colorado, USA), an immunoturbidimetric assay for the quantitative determination of HA in human serum. The unit of measurement was in ng/ml.

Statistical analysis:-
The Statistical Package for Social Sciences (SPSS) version 20.0 statistical software was used for data entry and analysis. Descriptive statistics of mean and standard deviation were calculated for all measurements taken. Independent t-test was used for the between group comparisons for mean scores of all parameters while Pearson's coefficient was used to calculate correlations. Alpha level for all statistics employed was set at $p < 0.05$. Log transformation of data was performed. Reference intervals for serum HA for the various groups were obtained after back transformation of the calculated values obtained using the formula: reference interval = m - 2s to m + 2s for a variable that follows a normal distribution where "m" was the mean and "s" was the standard deviation for the variable.

Results:-
The males were significantly taller ($t=6.81$; $p<0.01$) and heavier ($t=5.80$; $p<0.01$) than the females. The males also had significantly higher level of serum HA than their female counterparts ($t=2.22$; $p=0.03$). There were no age and BMI differences between the male and female participants ($p>0.05$) (table 1). Group 1 participants (older group) had significantly higher weight ($t=2,87$; $p=0.01$), BMI ($t=3.44$; $p<0.01$) and serum HA ($t=9.49$; $p<0.01$) than those in group 2 (younger group) (table 2). In each group, there was no significant difference in the age of male and female participants ($p>0.05$). However, male participants were significantly heavier and taller than their female counterparts. In group 1 female participants had significantly higher BMI values ($p<0.05$). Furthermore, male participants had higher serum HA than female participants ($t=2.55$; $p=0.02$). (table 3 and 4).

In each group, participants' serum HA level had significant positive correlation with their age ($p<0.05$) but had no significant correlation with their height and weight ($p>0.05$). In group 1, serum HA had significant negative correlation with BMI ($r=-0.34$; $p=0.03$) (table 5).

In group 1 the reference interval for serum HA level for the males and females were 19.44 - 25.87ng/ml and 19.55-23.35ng/ml respectively. For group 2, the reference interval for the males and females were 15.12 - 23.10ng/ml and 13.42 - 21.96ng/ml respectively. The upper reference limits were higher in group 1 than in group 2. In both groups, the upper reference limits were higher in the males than in the females (table 6).

Discussion:-
Hyaluronic acid is one of the biomarkers that can be used for the diagnosis of early stage KOA even though so far, it has not entered routine clinical use. Levels of serum HA are able to differentiate between healthy individuals without KOA and patients with KOA. It is also capable of differentiating between various severities of KOA. The diagnosis of KOA is usually made late based upon clinical and radiological (K/L Classification) criteria when joint

tissue degeneration is already advanced beyond the point at which pharmacological or surgical interventions can delay or reverse the process. For this reason, research focus has shifted to biomarkers like HA which are capable of detecting the disease at the early stage. Some researchers have reported increased production and release of HA from arthritic joints which is thought to reflect the localized inflammation occurring in the synovial lining (Engstrom-Laurent and Hallgren, 1985; Poole et al, 1990) and cartilage degradation (Hedin 1991). The increased HA content in inflamed synovium has also been suggested to contribute to the joint stiffness and edema seen in arthritis (Wells et al, 1992). Serum HA and KOA have also been linked in several previous studies. One study reported that baseline serum HA levels were predictive of arthroscopically determined tibiofemoral joint space disease progression in a 1-year follow-up of patients with KOA (Georges et al, 1997), and another reported a positive correlation between serum HA levels and the degree of joint space narrowing in KOA (Sharma et al, 1998). These associations with KOA in varying stages of the disease process suggest that HA may be useful as a biomarker for making early diagnosis of KOA.

This study showed that the serum HA levels for the males were significantly higher than those of the females in both groups. This gender bias in serum HA levels between the males and females had been precviously reported in the Johnston County Osteoarthritis Project (Elliott et al, 2005). However, Singh et al (2014) reported no difference in HA levels between the male and female gender. In the present study, unlike in group 2, there was a significant negative correlation between serum HA and BMI in group 1. There was no significant correlation between serum HA and anthropometric measurements of height and weight in any of the groups. Similar results had been previously reported (Elliott et al, 2005; Turan et al, 2007).

The was a positive significant correlation between levels of serum HA and age of the participants in each group. The correlation was however stronger in group 1 than in group 2. Similar correlations between HA and age have been reported in other studies (Elliott et al, 2005; Sdhir et al, 2014; Inoue et al, 2011). One of the reasons for the increase in HA levels with age is that the molecular weight and size of HA in cartilage decreases with age, but the total amount increases (Holmes et al, 1988). Some previous studies have also shown that serum HA level may be influenced by some factors other than the KOA disease process itself. Such factors include the activity level, eating and intake of supplements for the knee which are more likely in older people. These factors have been reported to increase serum HA levels (Manicourt et al, 2006; Criscione et al, 2005; Hinghofer-Szalkay et al, 2002; Rossler et al, 1998).

The differences in serum HA levels for the different age groups and genders in the present study suggests that age and gender should be considered in establishing reference intervals for HA to make the values more clinically precise and useful for interpreting laboratory results. Levels of serum HA above the upper reference limits for a particular age group and gender after evaluation of other clinical and laboratory findings may indicate early KOA in the absence of radiological features. Early diagnosis may open the door for early intervention. Also, early commencement of preventive measures in those whose serum HA is above the upper limit of the reference interval that have not developed structural changes may prevent development of KOA.

Conclusion:-

Hyaluronic acid can play a crucial role in diagnosis of early KOA because of its significant association with the disease. The researchers propose that this study be replicated in many centers in Nigeria using larger sample sizes. Once the reference intervals are validated, it can be introduced into routine clinical practice.

Acknowledgement:-

None.

Author Contributions:-

Chima Ihegihu and Ebere Ihegihu conceived and designed the study. Chika Anumba performed the HA analyses. All the authors collected and assembled the data. Emmanuel Okoye and Kenneth Ani performed the statistical analysis and interpretation. Chima Ihegihu and Ebere Ihegihu, drafted the manuscript. All the authors critically revised the manuscript for intellectual content, read and approved the final version for submission.

Funding:-

No external funding was obtained for the study.

Conflict Of Interest:-
None declared.

Ethical Approval:-
The study was approved by the ethical committee of the institution.
"We represent that this submission is original work, and is not under consideration for publication with any other journal."

Table 1:- Physical characteristics of the participants

	Gender	**N**	**Mean**	**Standard Deviation**	**T-value**	**P-value**
Age (years)	Male	40	31.83	10.25	0.01	0.99
	Female	40	31.80	10.08		
Height (m)	Male	40	1.82	0.059	6.81	<0.01*
	Female	40	1.73	.05		
Weight (Kg)	Male	40	77.78	4.04	5.80	<0.01*
	Female	40	72.40	4.25		
BMI (Kg/m^2)	Male	40	23.61	.98	-2.26	0.27
	Female	40	24.13	1.08		
HA (ng/ml)	Male	40	20.64	2.59	2.22	0.03*
	Female	40	19.34	2.64		

Table 2:- Comparison of the physical characteristics of the two groups (group1: participants 30years and above; group2: participants 29years and below)

	Groups	**N**	**Mean**	**Standard Deviation**	**t-value**	**p-value**
Age(years)	Group 1	40	41.26	4.36	25.04	<0.01*
	Group 2	40	22.35	1.97		
Height (m)	Group 1	40	1.78	.06	0.60	0.55
	Group 2	40	1.77	.07		
Weight (Kg)	Group 1	40	76.60	3.48	2.87	<0.01*
	Group 2	40	73.58	5.69		
BMI (Kg/m^2)	Group 1	40	24.25	1.15	3.45	<0.01*
	Group 2	40	23.49	.80		
HA (ng/ml)	Group 1	40	21.94	1.46	9.49	<0.01*
	Group 2	40	18.04	2.15		

Table 3:-Comparison of the physical characteristics of the males and females in group1 (those 30years and above).

	Gender	**N**	**Mean**	**Standard Deviation**	**T-value**	**P-value**
Age (years)	Male	20	41.35	4.61	0.108	0.92
	Female	20	41.20	4.20		
Height (m)	Male	20	1.82	0.06	4.773	<0.01*
	Female	20	1.74	0.04		
Weight (Kg)	Male	20	78.30	3.31	3.51	<0.01*
	Female	20	74.90	2.79		
BMI (Kg/m^2)	Male	20	23.73	1.03	-3.198	<0.01*
	Female	20	24.77	1.04		
HA (ng/ml)	Male	20	22.49	1.67	2.546	.02*
	Female	20	21.39	0.97		

Table 4:- Comparison of the physical characteristics of the males and females in group 2 (those 29 years and below).

	Gender	**N**	**Mean**	**Standard Deviation**	**T-value**	**P-value**
Age (years)	Male	20	22.30	1.81	-0.16	0.88
	Female	20	22.40	2.16		
Height (m)	Male	20	1.81	0.06	4.79	<0.01*
	Female	20	1.73	0.06		
Weight (Kg)	Male	20	77.25	4.68	5.33	<0.01*
	Female	20	69.90	4.01		
BMI (Kg/m^2)	Male	20	23.49	0.93	0.03	0.98
	Female	20	23.49	0.66		
HA (ng/ml)	Male	20	18.79	1.95	2.33	0.03*
	Female	20	17.29	2.12		

KEY:
*= Significant at $p<0.05$

Table 5:- Pearson's Correlation test for serum HA levels with physical characteristics of the participants

	Age	**Height**	**Weight**	**BMI**
Group 1(30years and older)				
r	0.76	0.30	0.12	-0.34
P-value	<0.01*	0.06	0.48	0.03*
Group 2(29years and younger)				
r	0.42	0.11	0.16	0.10
P-value	0.01*	0.49	0.31	0.55

KEY:*= Significant at $p<0.05$

Table 6:-Reference intervals of serum HA for the different gender in each age group

	Gender	**Reference Interval(ng/ml)**
Group 1(30years and older)	Male	19.44 – 25.87
	Female	19.55 – 23.35
Group 2(29years and younger)	Male	15.12 – 23.10
	Female	13.42 – 21.96

References:-

1. Fraser, J.R., Laurent, T.C., Laurent, U.B. (1997). Hyaluronan: its nature, distribution, functions and turnover. *J. Intern. Med*., 242 (1): 27–33.
2. Garnero, P., Rousseau, J.C., Delmas, P.D. *(2000).* Molecular basis and clinical use of biochemical markers of bone, cartilage, and synovium in joint diseases. *Arthritis Rheum.*, 43*:* 953–68.
3. Holmes, M.W., et al. (1988). Hyaluronic acid in human articular cartilage. Age-related changes in content and size. *Biochem*., J. 250 (2): 435–441.
4. Biomarkers Definitions Working Group (2001). Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clin Pharmacol Ther*., 69:89–95.
5. Frank, R., Hargreves, R. (2003). Clinical biomarkers in drug discovery and development. *Nat Rev Drug Discovery*., 2:567–80.
6. Rousseau, J.C., Pierre, D.D. (2007). Biological markers in osteoarthritis. *Nat Clinpra rheum.*, 3(6):346–56.
7. Kane, E.D. (2007). Biomarkers aid early detection of joint disease, bone damage. *DVM newsmagazine*., 24:46–49.
8. Turan, Y., Bal, S., Gurgan, A., Topac, H., and Koseoglu, M. (2007). Serum hyaluronan levels in patients with knee osteoarthritis. *Clin Rheumatol*., 26: 1293–1298.
9. Garnero, P., Rousseau, J.C., Demas, P.D. (2000). Molecular basis and clinical use of biochemical marker of bone, cartilage, and synovium in joint disease. *Arthritis Rheum*., 43:953–68.

10. Davies-Tuck, M.L., Wluka, A.E., Wang, Y., Teichtahl, A.J., Jones, G., Ding, C. et al. (2008). The natural history of cartilage defects in people with knee osteoarthritis. *Osteoarthritis Cartilage*., 16: 337–342.
11. Kamei, G., Sumen, Y., and Sakaridani, K. (2008). Evaluation of cartilage defect at medial femoral condyle in early osteoarthritis of the knee. *Magn Reson Imaging*., 26: 567–571.
12. Lee, C.L., Huang, M.H., Chai, C.Y., Chen, C.H., Su, J.Y., and Tien, Y.C. (2008). The validity of in vivo ultrasonographic grading of osteoarthritic femoral condylar cartilage: a comparison with in vitro ultrasonographic and histologic gradings. *Osteoarthritis Cartilage*., 16: 352–358.
13. Wollheim F.A. *(1999).* Serum markers of articular cartilage damage and repair. *Rheum Dis Clin North Am.,* 25*:* 417–32.
14. Elliott, A.L., Kraus, V.B., Luta, G., Stabler, T., Renner, J.B., Woodard, J. et al. (2005). Serum hyaluronan levels and radiographic knee and hip osteoarthritis in African Americans and Caucasians in the Johnston County Osteoarthritis Project. *Arthritis Rheum*., 52: 105–111.
15. Turan, Y., Bal, S., Gurgan, A., Topac, H., Koseoglu, M. (2007). Serum hyaluronan levels in patients with knee osteoarthritis. *Clin Rheumatol*., 26(8):1293–98.
16. Sharif, M., George, E., Shepstone, L., Knudson, W., Thonar, E.J., Cushnaghan, J. et al. (1995). Serum hyaluronic acid level as a predictor of disease progression in osteoarthritis of the knee. *Arthritis Rheum*., 38: 760–767.
17. Pavelka, K., Forejtová, S., Olejárová, M., Gatterová, J., Senolt, L., Spacek, P. et al. (2004). Hyaluronic acid levels may have predictive value for the progression of knee osteoarthritis. *Osteoarthritis Cartilage*., 12: 277–283.
18. Kellgren J.H., Lawrence, J.S (1963). *The epidemiology of chronic rheumatism, atlas of standard radiograph*s. Oxford*: Blackwell Scientific.*
19. Engstrom-Laurent, A., Hallgren, R. *(1985).* Circulating hyaluronate in rheumatoid arthritis: relationship to inflammatory activity and the effect of corticosteroid therapy. *Ann Rheum Dis.,* 44*:* 83–8.
20. Poole, A.R., Witter, J., Roberts, N., Piccolo, F., Brandt, R., Paquin, J., *et al. (1990).* Inflammation and cartilage metabolism in rheumatoid arthritis: studies of the blood markers hyaluronic acid, orosomucoid, and keratan sulfate. *Arthritis Rheum.,* 33*:* 790–9.
21. Hedin, P.J., Weitoft, T., Hedin, H., Engstrom-Laurent, A., Saxne, T. *(1991).* Serum concentrations of hyaluronan and proteoglycan in joint disease: lack of association. *J Rheumatol.,* 18*:* 1601–5.
22. Wells, A.F., Klareskog, L., Lindblad, S., Laurent, T.C. *(1992).* Correlation between increased hyaluronan localized in arthritic synovium and the presence of proliferating cells: a role for macrophage-derived factors. *Arthritis Rheum.,* 35*:* 391–6.
23. Georges, C., Vigneron, H., Ayral, X., Listrat, V., Ravaud, P., Dougados, M,, *et al. (1997).* Serum biologic markers as predictors of disease progression in osteoarthritis of the knee *[letter]*. *Arthritis Rheum.,* 40*:* 590–1.
24. Sharma, L., Hurwitz, D.E., Thonar, E.J., Sum, J.A., Lenz, M.E., Dunlop, D.D., *et al. (1998).* Knee adduction moment, serum hyaluronan level, and disease severity in medial tibiofemoral osteoarthritis. *Arthritis Rheum.,* 41*:* 1233–40.
25. Singh, S., Kumar, D., Sharma, N.R. (2014). Role of Hyaluronic Acid in Early Diagnosis of Knee Osteoarthritis. *Clin Diagn Res*., 8(12).
26. Inoue, R., Ishibashi, Y., Tsuda, E., Yamamoto, Y., Matsuzaka, M., Takahashi, I., Danjo, K., Umeda, T., Nakaji, S., Toh, S. (2011). Knee osteoarthritis, knee joint pain and aging in relation to increasing serum hyaluronan level in the Japanese population. *Osteoarthritis Cartilage*., 19(1):51-7.
27. Manicourt, D.H., Azria, M., Mindeholm, L., Thonar, E.J., and Devogelaer, J.P. (2006). Oral salmon calcitonin reduces Lequesne's algofunctional index scores and decreases urinary and serum levels of biomarkers of joint metabolism in knee osteoarthritis. *Arthritis Rheum*., 54: 3205–3211.
28. Criscione, L.G., Elliott, A.L., Stabler, T., Jordan, J.M., Pieper, C.F., and Kraus, V.B. (2005). Variation of serum hyaluronan with activity in individuals with knee osteoarthritis. *Osteoarthritis Cartilage*., 13: 837–840.
29. Hinghofer-Szalkay, H.G., Mekonen, W., Rössler, A., Schwaberger, G., Lamprecht, M., and Hofmann, P. (2002). Post-exercise decrease of plasma hyaluronan: increased clearance or diminished production?. *Physiol Res.*, 51: 139–144
30. Rössler, A., László, Z., Kvas, E., and Hinghofer-Szalkay, H.G. (1998). Plasma hyaluronan concentration: no circadian rhythm but large effect of food intake in humans. *Eur J Appl Physiol Occup Physiol*., 78: 573–577.