Diagnostic performance of bone metabolic indexes for the detection of stroke

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ABSTRACT

الأهداف: استكشاف أداء تشخيص 25 هيدروكسي فيتامين د وهرمون الغدة الدرقية (PTH) وانزيم الفوسفاتاز القلوي من العظام (BALP)، وأوستيوكالسين (OC) في توقع حدوث السكتة الدماغية.

الطريقة: أجريت هذه الدراسة بأثر رجعي في المستشفى الثاني التابع لجامعة نانتشانغ، نانتشانغ، مقاطعة جيانغشي، الصين. تضمن مرضى المجموعات التجريبية 121 مريض مصاب بالاحتشاء الدماغي و103 مصاب بنزيف فى المخ. و100متطوع في المجموعة المرجعية و80 مصاب برضه دماغيه في المجموعة المرجعية المرضية جرى قياس 25 هيدروكسي فيتامين د وPTH وBALP وOC باستخدام مقايسة مناعية إنزيمية.

النتائج: كان تركيز مصل 25 هيدروكسي فيتامين د في مرضى السكتة الدماغيه أقل بشكل ملحوظ من المجموعة مرجعية (p<0.05) بينما كان نقصه لديهم أعلى بكثير من مجموعة مرجعية (p<0.05). تجاوزت تركيزات مصل PTH وOC في مرضى السكتة الدماغية تلك التي وُجدت في المجموعات مرجعية (p<0.05). وكان المستوى غير الطبيعي لدى مرضى السكتة الدماغية أعلى بكثير من المجموعة المرجعية و زادت تركيزات BALP في مرضى الاحتشاء الدماغي كثيراً مقارنة بالمجموعة مرجعية. بالإضافة إلى ذلك، عُثر على مستويات غير طبيعية من BALP في مرضى السكتة الدماغية أعلى من المستويات الموجودة في المجموعة المرجعية ومع ذلك، لم يتم العثور على تركيزات ومستويات غير طبيعية من BALP في المرضى الذين يعانون من نزيف فى المخ لتكون مختلفة كثيراً عن تلك التي وُجدت في مرضى الاحتشاء الدماغي والمجموعات المرجعية ولم هناك اختلافات كبيرة بين المجموعتين المرجعية.

الخاتمة: يشير نقص 25 هيدروكسي فيتامين د والزيادة المفرطة في PTH وBALP وOC إلى ارتفاع مخاطر السكتة الدماغية.

Objectives: To explore the diagnostic performance of 25-hydroxyvitamin D (25(OH)D), parathyroid hormone (PTH), bone alkaline phosphatase (BALP), and osteocalcin (OC) in predicting stroke.

Methods: This retrospective survey was conducted in The Second Affiliated Hospital to Nanchang University, Nanchang, Jiangxi Province, China. involved 121 cerebral infarction patients and 103 cerebral hemorrhage patients as the experimental groups, 100 volunteers as the healthy control group and 80 brain trauma patients as the disease control group. The 25(OH)D, PTH, BALP, and OC levels of all participants were measured by electrochemiluminescence immunoassay.

Results: The serum concentration of 25(OH)D in stroke patients was appreciably lower than that of the control groups (p<0.05), and subsequently, the deficiency level of 25(OH)D in the stroke population was considerably higher than that of the control groups (p<0.05). The serum concentrations of PTH and OC in stroke patients exceeded those found in the control groups (p<0.05), and the abnormal level in the stroke patients was also higher than that of the control. Compared with the control group, BALP concentrations in cerebral infarction patients were increased significantly. Additionally, abnormal levels of BALP in stroke patients were found to be higher than those in the control groups. However, concentrations and abnormal levels of BALP in cerebral hemorrhage patients were not found to be significantly different than those found in cerebral infarction and the control groups, There were no substantial differences between the 2 control groups.

Conclusion: Lack of 25(OH)D and excessive PTH, BALP, and OC could indicate a high risk of stroke.

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Stroke, including ischemic stroke (also known as cerebral infarction) and cerebral hemorrhage, is a series of cerebrovascular diseases, which are characterized by sudden onset, which can result in partial or dispersed rapid cerebral function damage. Cerebral infarction occurs when the dependent vessels are disturbed. This can lead to partial or whole ischemia, hypoxia, and necrosis and is relevant to neurologic impairment in the brain. Atherosclerosis, in particular, is one of the common causes of stroke. Cerebral hemorrhage is bleeding in the brain parenchyma except cerebral trauma. The most common cause is a vascular tear due to sudden elevated blood pressure resulting from hyaline degeneration and fibrinoid necrosis in cerebral arterioles, which is caused by long-term hypertension. Stroke is a leading cause of death in China. Since stroke demonstrates a high morbidity and mortality rate, exploring its mechanisms can be effective in its prevention. 25-hydroxyvitamin D [25(OH)D], parathyroid hormone (PTH), bone alkaline phosphatase (BALP), and osteocalcin (OC) were often applied in the detection of bone generation and metabolism and the treatment of osteoporosis. Recently, bone metabolic indexes or their products have been found in myocardial cells, vascular cells, smooth muscle cells, endothelial cells, and so forth.[1] However, the correlation between bone metabolic indexes and the risk and prognosis of stroke is still unclear. Recently, several prospective experiments showed that long-lasting low 25(OH)D level may indicate a high risk of stroke.[2] Deficiency of 25(OH)D may predict a worse prognosis of high mortality.[3] Conversely, other experiments reported no observed correlation between 25(OH)D level and the occurrence of stroke.[4] A similar conflict was found in studies of the relationship of the other metabolic indexes in stroke patients.[5] In brief, the current studies investigated the diagnostic performance of 25(OH)D, PTH, BALP, and OC measurements in predicting stroke are limited due to the sample number and the length of time needed for follow-up. Compared with the previous studies in this field, the presented results include a larger patient population and offer a better evaluation of the predictive value of 25(OH)D, PTH, BALP, and OC levels in the diagnosis of stroke.

Methods.

Patients. In this retrospective study, all 121 patients with cerebral infarction were between 39 and 84 years of age with the average age being 62.46 years and with a standard deviation (SD) of 4.72 (62.46 ± 4.72). The 103 cerebral hemorrhage patients were aged from 32 to 85 years with an SD of 4.78 (55.80 ± 4.78). Both experimental groups were selected from patients diagnosed between January 2014-December 2015 in The Second Affiliated Hospital to Nanchang University, Nanchang, Jiangxi Province, China. The control groups consisted of 80 brain trauma patients aged from 20 to 86 years (49.80 ± 10.76) and 100 healthy volunteers aged from 30 to 84 years (59.12 ± 10.01). This study was approved by the ethics committee of The Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi Province, China, and was in accordance with the Declaration of Helsinki. The inclusion criteria for the control groups included the absence of clinical cerebral infarction symptoms and no foci found by cranial computed tomography (CT) or magnetic resonance imaging (MRI).

The inclusion criteria of the experimental groups were as follows: 1) age >18 years, 2) accordance with the diagnostic criteria of 2013 USA Acute Cerebral Infarction Guidelines,[6] and 3) presence of cerebral infarction as demonstrated by CT or MRI. The exclusion criteria used in this study: 1) evidence of osteoporosis or other diseases of abnormal bone metabolism, and 2) ingestion/injection of vitamin D or any drugs that may have influenced bone metabolism within 3 months of the study's start.

Material. Due to the rapid progress of cerebral hemorrhage, samples were collected within 6 hours of its occurrence. Cerebral infarction samples were collected within 24 hours because of the slower progress. Brain trauma samples were collected within 2 hours. The fasting blood samples were collected without an anticoagulant, and the serum was acquired by centrifugation for 15 minutes at the speed of 3000 rpm. Serum 25-(OH)D, PTH, and OC were measured by electrochemiluminescence immunoassay (ECLIA) in the Cobas e 601 (Roche Company, Shanghai, China). The BALP level was measured by JY-Color-BAP AB (JSY, Beijing China) following the manufacturer's recommendations. Bone alkaline phosphatase and all measurement processes were operated blindly according to the standard operating procedure (SOP) files in the clinical laboratory.

Comparison criteria. The evaluation of the levels of 25(OH)D is in accordance with the international criteria.[7] The 25(OH)D deficiency is <20 ng/ml, insufficiency is 20-29 ng/ml, and sufficiency is >30 ng/ml. According to the parathyroid hormone detection kit, a value between 15 pg/ml and 65 pg/ml is considered normal; all other values are considered abnormal. For osteocalcin, the normal range is between

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11 ng/ml and 48 ng/ml. The bone alkaline phosphatase detection kit shows that lower than 150 u/l is normal in pregnancy, less than 200 u/l is normal for children, and lower than 100 u/l is normal for the other adults.

Statistical methods. The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 17 was used for the statistical analysis of solution 25-(OH)D, PTH, BALP, and OC. The normality of data distribution was checked by the Kolmogorov-Smirnov test. Enumeration data were described in frequency and percentage; χ^2 test was used to compare classification of bone metabolic indexes between groups; continuity correction was used when the variate was <5. Measurement data in accordance with normal distribution are described in mean and standard deviation (Mean ± SD). The T test was used to compare the concentration of bone metabolic indexes between groups. A value of $p<0.05$ was considered statistically significant.

Results. In this study, a total of 224 stroke cases (121 cerebral infarction patients and 103 cerebral hemorrhage patients) and 180 controls (100 healthy individuals and 80 brain trauma patients) were selected according to the inclusion and exclusion criteria. The levels of 25-(OH)D, PTH, BALP, and OC in the 4 groups are shown in **Table 1**. Significant difference was observed in the cases and controls ($p<0.05$). However, There is no significant difference in inter healthy groups or experimental groups ($p>0.05$). Serum 25-(OH)D in stroke group was definitely lower than control group, however PTH, BALP, and OC is on the contrary. **Table 2** shows that circulating concentrations of 25-(OH)D, PTH, BALP, and OC in the 2 groups have significant difference ($p<0.05$). The deficiency levels of 25-(OH)D of the experimental group was significantly higher than in the control group. Meanwhile abnormal level of PTH, BALP, OC was higher than in the control group. Moreover, **Table 3** compensatory shows that significant difference also existed between cerebral infarction and cerebral hemorrhage in BALP ($p<0.05$), and between cerebral infarction and control groups ($p<0.05$), however there is no significant difference between cerebral hemorrhage and control groups ($p>0.05$). parathyroid hormone, 25-hydroxyvitamin D,

Table 1 - The levels of 25-(OH)D, PTH, BALP, and OC in the 4 groups.

Disease cohort	25-(OH)D	PTH	BALP	OC
Cerebral infarction	15.48 ± 5.67*†	62.32 ± 4.37*†	97.54 ± 12.19*†	29.77 ± 9.54*†
Cerebral hemorrhage	15.53 ± 6.78*†	61.32 ± 3.24*†	87.29 ± 23.67	30.89 ± 4.57*†
Brain trauma	20.24 ± 8.91	47.07 ± 21.74	84.28 ± 6.35	25.52 ± 7.36
Healthy control	26.73 ± 3.70	47.29 ± 5.71	83.48 ± 53	24.78 ± 3.65

*Comparison with healthy control group, $p<0.05$, †compared with brain trauma $p<0.05$, 25-(OH)D - 25-hydroxyvitamin D, PTH - parathyroid hormone, BALP - bone alkaline phosphatase, OC - osteocalc

Table 2 - Classification of 25-(OH)D, PTH, BALP, and OC in experiment group and control group.

Variable	Experiment (n=180)	Control (n=224)
	n (%)	
25-(OH)D		
Sufficiency	39 (21.7)§	12 (5.4)
Insufficiency	99 (55.0)§	45 (20.1)
Deficiency	42 (23.3)§	167 (74.6)
BALP		
Normal	14 (7.8)§	53 (23.7)
Abnormal	166 (92.2)§	171 (76.3)
PTH		
<15 pg/ml or >65 pg/ml	4 (2.2)§	70 (31.3)
15 pg/ml-65 pg/ml	176 (97.8)§	154 (68.8)
OC		
<11 ng/ml or >48 ng/ml	5 (2.8)§	24 (10.7)
11 ng/ml-48 ng/ml	175 (97.2)§	200 (89.3)

§Comparison with control group, $p<0.05$, 25-(OH)D - 25-hydroxyvitamin D, PTH - parathyroid hormone, BALP - bone alkaline phosphatase, OC - osteocalc

Table 3 - Classification of 25-(OH)D, PTH, BALP, and OC in cerebral infarction, cerebral hemorrhage, Brain trauma, and healthy control group.

Variable	Control (n=224)		Experiment (n=180)	
	Cerebral infarction (n=121)	Cerebral hemorrhage (n=103)	Cerebral trauma (n=80)	Healthy control group (n=100)
	n (%)		n (%)	
25-(OH)D				
Sufficiency	7 (5.8)*†	5 (4.9)*†	10 (12.5)	29 (29.0)
Insufficiency	26 (21.5)*†	19 (18.5)*†	44 (55.0)	55 (55.0)
Deficiency	88 (72.7)*†	79 (76.7)*†	26 (32.5)	16 (16.0)
BALP				
Normal	36 (29.8)*†	17 (16.5)‡	3 (2.5)	11 (11.0)
Abnormal	85 (70.3)*†	86 (83.5)‡	77 (97.5)	89 (89.0)
PTH				
<15 pg/ml or >65 pg/ml	42 (34.7)*†	28 (27.2)*†	2 (2.5)	2 (2.0)
OC				
15 pg/ml~65 pg/ml	79 (65.3)*†	75 (72.8)*†	78 (97.5)	98 (98.0)
<11 ng/ml or >48 ng/ml	12 (9.9)*†	12 (11.7)*†	2 (2.5)	3 (3.0)
11 ng/ml~48 ng/ml	109 (90.1)*†	91 (88.5)*†	78 (97.5)	97 (97.0)

*Comparison with healthy control group $p<0.05$, †Comparison with brain trauma $p<0.05$, ‡Comparison with cerebral infarction, $p<0.05$, 25-(OH)D - 25-hydroxyvitamin D, PTH - parathyroid hormone, BALP - bone alkaline phosphatase, OC - osteocalc

and osteocalcin has no significant difference between cerebral infarction and cerebral hemorrhage ($p>0.05$). There were no difference between the 2 control groups ($p>0.05$).

Discussion. It is well known that 25(OH)D, PTH, BALP, and OC interact with each other in bone metabolism. Recently, it has been found that 25(OH)D, PTH, BALP, and OC also play an important role in vascular calcification.[8] This study shows decreased concentration of 25(OH)D, PTH, BALP, and OC when stroke occurred, which means detecting and adjusting the concentration maybe meaningful for stoke prevention and treat. Vitamin D is not equipped with biological activity in vivo, and its active type is $25(OH)_2D_3$.

Due to the short half-life of $25(OH)_2D_3$, vitamin D concentration is typically monitored by detection of serum 25(OH)D concentration. According to traditional opinions, vitamin D mainly exists in bones and kidneys. In recent years vitamin D has also been found in cardiac muscle cells, vascular smooth muscle cells, and endothelial cells.[1] Some scholars have proven that long-term low 25(OH)D concentrations accelerate the occurrence of stroke by some mechanism through prospective research. Sun et al[2] proved that low concentration may be a risk factor for stroke. Carrelli et al[9] discovered that low level 25(OH)D was related to increasing endarterial and carotid atherosclerosis plaque, and Chaudhuri et al[10] believed that 25(OH)D resulted in cardiovascular and cerebrovascular disease by affecting the atherosclerotic formation. Blondon et al's[11] research, however; showed that 25(OH)D affected the pathogenesis of heart and cerebral vessels through other approaches. Meanwhile, Daubial et al[12] indicated a correlation between 25(OH)D and stroke prognosis. On the contrary, some scholars did not think 25(OH)D and stroke had correlation.[4] In our retrospective research, the serum concentration of 25(OH)D in stroke patients was obviously lower than that of the control groups ($p<0.05$), and the deficiency levels of 25(OH)D in the stroke group was significantly higher than that in control group ($p<0.05$). This indicates that when stroke occurs, serum 25(OH)D concentration declines. Combined with the prospective research, this present study suggests that 25(OH)D may be a risk factor for stroke.

Recently BALP has been found in vascular cells, such as smooth muscle cells. Experiments show that BALP is linked to heavy drinking,[13] obesity, and high blood pressure,[14] and can catalyze the hydrolysis of pyrophosphate to weaken its ability to inhibit vascular calcification.[13] Some scholars believe that increased BALP is related to cardiovascular disease and stroke

because of vascular calcification, immune disorders, and atherosclerosis.[15] Meanwhile, some experts believe that increased serum BALP and serum phosphate are predictors of mortality after stroke.[16] The secretion of BALP and the rising background have a similarity with PTH. The data from Shimizu et al[17] showed that abnormal BALP levels give rise to atherosclerosis, and a high BALP level is correlated with ischemic stroke in males and hemorrhagic stroke in females while a low BALP level is correlated with the occurrence of all types of stroke in males and females. In the current study, BALP concentrations and abnormal levels were higher in cerebral infarction patients when compared with the control group. However, BALP concentrations and abnormal levels in cerebral hemorrhage patients were insignificant when compared with the control group. In view of the conclusion of Shimizu et al,[17] the BALP change may be different in different stroke types and different genders. In general, BALP levels change during the occurrence and progress of stroke. This suggests that stroke can be predicted and therapeutic intervention can be administered by adjusting the concentration of BALP. After adjusting for demographic and life style factors, a one year cross-sectional study refuted the relationship between elevated PTH levels and subclinical cerebrovascular disease.[18] Other studies have shown that primary hyperparathyroidism with elevating PTH and significantly increasing blood pressure heightened the risk of female cerebral infarction but had no effect on males. After parathyroidectomy, blood pressure was reduced to normal levels and the risk of stroke decreased.[19] In the meantime, other studies have shown that PTH isolated increased the pressure, which enhance damage effects on blood vascular leading to improve the incidence of cerebral infarction due to the vascular sclerosis disease.[20,21] An increasing concentration of PTH is one of the high risk factors of cardiovascular events, especially for patients with renal failure.[22,23] In the current study, serum concentration of PTH in stroke patients exceeded that of the control groups ($p<0.05$). Abnormal levels of PTH in stroke patients were also higher than those in the control groups. There was no significant difference between the 2 control groups indicating that PTH increases during a stroke event. This retrospective study indirectly proved that PTH positively correlates with the incidence of stroke.

Osteocalcin is a noncollagenous protein secreted by osteoblasts following the peak period of mineralization. Recent experiments have shown that OC also exists in vascular smooth muscle cells,[24] and other research shows that OC also participates in adjusting vascular calcification.[25] From the study of glucose and fat metabolisms, some researchers have concluded that serum OC levels are a significant predictor of cardiovascular disease.[26] Some experiments were also conducted on studying the mechanism of OC in promoting vascular calcification. This research showed that both osteoblast and osteoclast cells of mice *in vitro* and *in vivo* might form pathogenic mechanisms in various blood vessels through some circular paths, such as vascular calcification, blood vessel atherosclerosis, and intimal hyperplasia.[24] In this study, OC was comparable with PTH. The importance that OC plays in calcification corresponded to the results obtained in the current study.

This study was limited by its small sample size and the absence of investigation of the relationship between women/men and abnormally high and low BALP. A larger sample size will be used in future research, and the relationship between BALP and gender will be explored further.

In conclusion, this study demonstrated that 25(OH)D, PTH, BALP, and OC levels were altered in stroke patients. So detecting and adjusting the concentration of bone metabolic indexes may be a feasible way to predict, give therapy intervention, and relieve stroke.

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Ethical Consent

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.