The association between pineal gland calcification and intracranial atherosclerotic disease in older adults

Asociación entre calcificaciones de la glándula pineal y enfermedad aterosclerótica intracraneal en adultos mayores

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Abstract

Background: This study assesses whether pineal gland calcification (PGC) – a surrogate for reduced endogenous melatonin production – is associated with significant stenosis of large intracranial arteries – a biomarker of intracranial atherosclerotic disease (ICAD).

Methods: Individuals aged ≥60 years enrolled in the Three Villages Study received head CT to assess PGC and MRA to estimate stenosis of large intracranial arteries. Multivariate logistic regression models were fitted to assess the association between PGC and ICAD, after adjusting for relevant confounders. Inverse probability of exposure weighting was used to estimate the effect of PGC on ICAD.

Results: A total of 581 individuals were enrolled. PGC and ICAD were associated in a fully-adjusted logistic regression model (p=0.032). Inverse probability of exposure weighting showed an estimate for the proportion of ICAD among those without PGC of 3.7% and the adjusted-effect coefficient was 5.7% higher among those with PGC (p=0.031).

Conclusions: PGC is associated with ICAD. Study results provide grounds for evaluating the role of melatonin deficiency in ICAD progression.

Keywords: Pineal gland calcification, intracranial atherosclerosis, stenosis of large intracranial arteries, melatonin; population study, older adults

Resumen

Antecedentes: Este estudio evalúa si las calcificaciones de la glándula pineal (CGP), un biomarcador de deficiencia de producción de melatonina endógena, se asocia con estenosis significativa de las arterias intracraneales grandes, un biomarcador de enfermedad aterosclerótica intracraneal.

Métodos: Individuos de 60 años o más enrolados en el "Estudio de las Tres Villas" fueron sometidos a TC craneal evaluar el CGP y MRA para estimar la estenosis de las arterias intracraneales de mediano calibre. Se computaron modelos de regresión logística multivariada para evaluar la asociación entre PGC y enfermedad aterosclerótica intracraneal, luego de ajustar por covariables de relevancia. Se utilizaron modelos de ponderación de probabilidad inversa de la exposición para estimar el efecto de PGC sobre enfermedad aterosclerótica intracraneal.

Resultados: Un total de 581 personas estuvieron enroladas en este estudio. PGC y enfermedad aterosclerótica intracraneal se asociaron en un modelo de regresión logística ajustado por todas las covariables investigadas (p=0,032). La ponderación de probabilidad inversa de la exposición mostró una estimación de la proporción de enfermedad aterosclerótica intracraneal entre los que no tenían CGP del 3,7% y el coeficiente de efecto ajustado fue un 5,7% más alto entre los que tenían CGP (p=0,031).

Conclusiones: La presencia de CGP se asocia a enfermedad aterosclerótica intracraneal. Los resultados del estudio proporcionan una base racional para evaluar el papel de la deficiencia de melatonina en la progresión de la enfermedad aterosclerótica intracraneal.

Palabras clave: Calcificación de la glándula pineal, aterosclerosis intracraneal, estenosis de arterias cerebrales de mediano calibre, estudio poblacional, adultos mayores

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Introduction

The pineal gland secretes melatonin, a neuroendocrine hormone that is mainly associated with the sleep-wake cycle control.[1] This hormone also has antioxidative, anti- inflammatory, cardioprotective and neuroprotective effects.[2,3] In addition, melatonin down-modulates the expression of several molecules involved in the development and progression of atherosclerosis.[4] However, clinical information of the relationship between pineal gland dysfunction and atherosclerosis is limited.[5,6] The pineal gland becomes calcified in a sizable proportion of otherwise healthy individuals. Inasmuch as calcified pineal tissue is inactive, the degree of pineal gland calcification (PGC) may be used as a surrogate for reduced melatonin secretion. Indeed, it has been shown that the volume of non-calcified pineal tissue correlates directly with the amount of 6-sulphatoxymelatonin excretion in urine.[7] There are no studies addressing the association between PGC and intracranial atherosclerotic disease (ICAD). Based on the premise that severity of PGC can be used as a proxy for pineal gland dysfunction (and, hence, reduced endogenous melatonin production), we conducted a population-based study aimed to assess whether severity of PGC is associated with significant stenosis of large intracranial arteries (a recognized biomarker of ICAD).

Methods

This study was conducted in individuals aged ≥60 years actively enrolled in the Three Villages Study. The population is exposed to 12 daily hours of sunlight all year-round. Inhabitants share ethnicity (Amerindian ancestry), socio-economic status, dietary habits, and an overall comparable cardiovascular health status.[8] Individuals who signed a comprehensive informed consent and have no contraindications for the practice of neuroimaging studies received a head CT scan and a MRA of the intracranial vasculature. The study was approved by Ethics Committee of Hospital-Clínica Kennedy, Guayaquil, Ecuador (FWA 00030727).

High resolution CT was used to assess presence and severity of PGC and MRA to estimate stenosis of large intracranial arteries (Figure 1). Neuroimaging studies were performed with a Philips Brilliance 64 CT scanner and a Philips Intera 1.5T MR scanner (Philips Medical Systems, Eindhoven, the Netherlands). For CT, slice thickness was 3mm with no gap between slices, and PGC were graded according to the maximal density in Hounsfield Units (HU) of the calcified portion of the gland and its calcified fraction. According to a previously detailed protocol, both scores were summed for a total degree of calcification graded as none, mild, and moderate-to-severe calcification.[9] MRAs were performed using a three-dimensional time-of-flight sequence. Significant stenosis of large intracranial arteries was measured by the use of the MRA-validated WASID method.[10] The presence of any stenosis ≥50% was considered an indicator of ICAD. CTs and MRAs were independently read by one neurologist and one neuroradiologist blinded to clinical information. Kappa coefficients for inter-rater agreement were 0.86 for the presence and severity of PGC, and 0.73 for significant stenosis of intracranial arteries; discrepancies were reviewed by consensus.

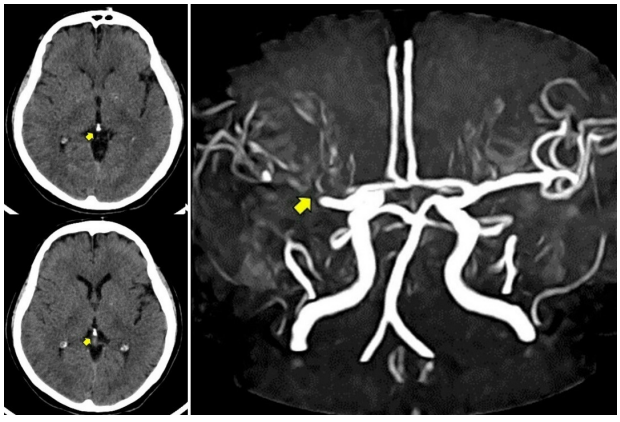

Figure 1. *Left panel:* Unenhanced CT of the head showing moderate-to-severe pineal gland calcification (arrows). *Right panel*: Time-of-flight magnetic resonance angiography of intracranial arteries showing significant asymptomatic stenosis of the right middle cerebral artery (arrow).

Demographics and cardiovascular risk factors were included as covariates and assessed following the American Heart Association (AHA) criteria: a poor smoking status was designated if the subject was a current smoker, a poor body mass index if ≥30 kg/m2, a poor physical activity if the subject engaged in no moderate or vigorous physical activity, a poor diet if the individual had 0-1 component of the AHA healthy diet, a high blood pressure if ≥140/90 mmHg, a poor fasting glucose if ≥126 mg/dL, and a poor total cholesterol levels if ≥240mg/dL.[11]

Data analyses were carried out by using STATA version 17 (College Station, TX, USA). In unadjusted analyses, continuous variables were compared by linear models and categorical variables by the x2 or Fisher exact test as appropriate. A multivariate logistic regression model was fitted to assess the independent association between PGC severity and significant stenosis of large intracranial arteries (as dependent variable). We utilized inverse-probability-weighted regression adjustment to obtain the exposure effect of PGC over stenosis of large intracranial arteries, by fitting a potential outcomes model with the exposure model being multinomial and the outcome being logistic.

Results

A total of 590 older adults received head CTs and MRAs of intracranial vessels. Nine of them were not included because motion/metal artifacts precluded proper

PGC readings. The mean age of the 581 participants was 71±8.4 years (median age: 69 years) and 332 (57%) were women. Twenty-one individuals (4%) were current smokers, 147 (25%) had a body mass index ≥30 kg/m2, 66 (11%) had poor physical activity, 55 (9%) had a poor diet, 250 (43%) had blood pressure ≥140/90 mmHg, 176 (30%) had fasting blood glucose ≥126 mg/dL, and 69 (12%) had total cholesterol levels ≥240 mg/dL. None of the study participants took or were currently taking melatonin pills for sleeping problems.

PGC were categorized as mild in 299 (51%) individuals and as moderate-to-severe in 174 (30%); the remaining 108 (19%) had no CT evidence of PGC. Significant stenosis of large intracranial arteries were noticed in 40 (7%) participants. Stenosis involved the anterior circulation in 24 cases (middle cerebral artery in 18, internal carotid artery in four, and anterior cerebral artery in two), the posterior circulation in 15 cases (posterior cerebral artery in 10 and basilar artery in five), and both the anterior and the posterior circulations in the remaining case.

Table 1 depicts characteristics of study participants across categories of PGC severity and stenosis of large intracranial arteries. In unadjusted analyses, both PGC and ICAD share some risk factors, such as being male and a smoker. Other covariates were only more frequent in individuals with significant stenosis of large intracranial arteries (increasing age and having a blood pressure ≥140/90 mmHg). Also in unadjusted analyses, 4/108 (4%) individuals without PGC, 17/299 (6%) with mild PGC, and 19/174 (11%) with moderate-to-severe PGC had significant stenosis of large intracranial arteries. A univariate logistic regression model showed a significant association between PGC (any calcification) and ICAD (OR: 1.87; 95% C.I.: 1.14 – 3.09; p=0.014). Another univariate logistic regression model, taking into account categories of PGC (none, mild, and moderate-to-severe), disclosed a significant association between moderate-to-severe PGC and ICAD (OR: 3.19; 95% C.I.: 1.05 – 9.64; p=0.040), but not between mild PGC and ICAD (OR: 1.57; 95% C.I.: 0.52 – 4.77; p=0.428). The latter model revealed a linear effect between PGC severity and ICAD.

When age and gender were added to the above-mentioned models, the association between PGC (any calcification) and ICAD remained significant (OR: 1.81; 95% C.I.: 1.09 – 2.99; p=0.021), as well as the association between moderate-to-severe PGC and ICAD (OR: 3.12; 95% C.I.: 1.01 – 9.59; p=0.047), but not between mild PGC and ICAD (OR: 1.65; 95% C.I.: 0.54 – 5.09; p=0.380). Age and gender were significant covariates at the p<0.02 level. The linear effect between PGC severity and ICAD was also signaled in these models.

A multivariate logistic regression model, adjusted for all the investigated covariates, showed a significant association between PGC (any calcification) and ICAD (OR: 1.76; 95% C.I.: 1.05 – 2.95; p=0.032). In this model, being male (p=0.043) and having high blood pressure (p=0.004) remained as significant covariates (Table 2).

The exposure-effect model also revealed a significant association between moderate-to-severe PGC (as the exposure) and ICAD (as the outcome), after adjusting for covariates associated with either the exposure or the outcome, or both. According to this model, the inverse probability of exposure weighting showed an estimate for the proportion of ICAD among those without PGC of 3.7% (95% C.I.: 2.2 – 7.1%; p=0.037) and an exposure-effect coefficient (measuring the difference between none and moderate-to-severe PGC) that was 5.7% higher than the exposure-effect proportion (β: 0.057; 95% C.I: 0.005 – 0.108; p=0.031).

Table 1. Characteristics of Atahualpa, El Tambo and Prosperidad residents aged ≥60 years across categories of pineal gland calcification and significant stenosis of large intracranial arteries (univariate analyses).

	Pineal gland calcification				Large intracranial artery stenosis		
Variable	None (n=108)	Mild (n=299)	Mod-severe (n=174)	p value	Non-significant stenosis (n=541)	Significant stenosis (n=40)	p value
Age, years (mean±SD)	71.4 ± 8.6	71.2 ± 8.4	70.4 ± 8.4	0.522	70.8 ± 8.3	74 ± 9.6	0.020*
Female gender, n (%)	66 (61)	186 (62)	80 (46)	0.002*	317 (59)	15 (38)	0.009*
Current smoker, n (%)	2 (2)	6 (2)	13 (7)	0.005*	18 (3)	3 (8)	0.016*
Body mass index ≥30 kg/m2, n (%)	30 (28)	83 (28)	34 (20)	0.113	141 (26)	6 (15)	0.120
Poor physical activity, n (%)	11 (10)	33 (11)	22 (13)	0.795	61 (11)	5 (13)	0.796
Poor diet, n (%)	12 (11)	22 (7)	21 (12)	0.195	49 (9)	6 (15)	0.215
Blood pressure ≥140/90 mmHg, n (%)	49 (45)	131 (44)	70 (40)	0.647	223 (41)	27 (68)	0.001*
Fasting glucose ≥126 mg/dL, n (%)	32 (30)	94 (31)	50 (29)	0.815	164 (30)	12 (30)	0.966
Total cholesterol ≥240 mg/dL, n (%)	10 (9)	34 (11)	25 (14)	0.405	64 (12)	5 (13)	0.899

* Statistically significant result.

Table 2. Logistic regression model showing the independent relationship between pineal gland calcification and significant stenosis of large intracranial arteries.

Significant arterial stenosis	Odds ratio	95% confidence interval	p value
Pineal gland calcification	1.76	1.05 – 2.95	0.032*
Age	1.04	0.99 – 1.08	0.067
Male gender	2.13	1.02 – 4.35	0.043*
Current smoker	1.91	0.48 – 7.62	0.359
Body mass index ≥30 kg/m2	0.63	0.25 – 1.62	0.340
Poor physical activity	0.81	0.28 – 2.35	0.697
Poor diet	1.54	0.58 – 4.11	0.386
Blood pressure ≥140/90 mmHg	2.88	1.40 – 5.80	0.004*
Fasting glucose ≥126 mg/dL	0.94	0.45 – 1.97	0.874
Total cholesterol ≥240 mg/dL	1.26	0.45 – 3.54	0.657

* Statistically significant result.

Discussion

Atherosclerosis is a pathological process that often remains clinically unnoticed for years. It often starts with dysfunction of the subendothelial layer, accumulation of lipids, and migration of white blood and smooth muscle cells, which is followed by foam cell formation, damage of the extracellular matrix, plaque progression and disruption.[12] Protective effect of melatonin against atherosclerosis is supported by the concept that melatonin modulates most of the above-mentioned steps of atherogenesis, and thus exerts a protective effect for the development and progression of atherosclerotic disease.[5] Among other mechanisms, melatonin has been shown to reduce the expression of reactive oxygen species, endothelin-1, tumor necrosis factor-α, interleukin-1β, interleukin-6, monocyte chemoattractant protein-1, interferon-γ, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, while upregulates the expression of endothelial nitric oxide synthases.[13] In addition, melatonin may contribute to reduce risk factors that have traditionally been associated with atherosclerosis.[14]

Clinical studies investigating the link between PGC and atherosclerosis are almost nil. To the best of our knowledge, only one observational study of patients with low-back pain disclosed a significant association between PGC and atherosclerosis of the abdominal aorta.[6] The potential association between PGC and ICAD has not been addressed, and results of the present study cannot be compared to others. Nevertheless, some reports are in line with results presented in this study, particularly the recognized relationship between PGC or melatonin deficiency and cardiovascular diseases, stroke, or neuro-degenerative disorders.[15] Our findings open new avenues for research on the role of PGC (and endogenous melatonin deficiency) in the development of ICAD, which is a leading cause of stroke worldwide.

This study has limitations that go beyond its cross-sectional design. We relied on the severity of PGC as a surrogate for melatonin deficiency but did not measure melatonin levels. In addition, the association between PGC and ICAD was evaluated in a population that is exposed to 12 hours of sunlight all year-round, and our results may not be generalizable to other geographical regions. On the other hand, the population-based design with unbiased selection of participants, together with the use of high-resolution CT for detection of PGC, the blinded imaging reading process with high inter-reader agreement and the results of multivariate and exposure-effect models, argue for the strength of our results.

In conclusion, study results disclose a significant independent association between PGC and ICAD. Further studies, preferably with a longitudinal design, should take into account serum levels of melatonin or its active metabolite 6-sulphatoxymelatonin in urine to confirm our findings.

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