Armando L. Morera-Fumero[a,b]
Estefanía Díaz-Mesa[a,c]
Pedro Abreu-González[d]
Lourdes Fernandez-Lopez[a,e]

Acute paranoid schizophrenia relapsed inpatients present summer/winter but not day/night changes in serum S100B concentrations

[a] Departamento de Medicina Interna, Dermatología y Psiquiatría, Facultad de Ciencias de la Salud, Universidad de La Laguna (ULL), La Laguna, Santa Cruz de Tenerife, Spain
[b] Consultoria Psiquiátrica SC, Santa Cruz de Tenerife, Spain
[c] Servicio de Psiquiatría, Hospital Universitario de Canarias, La Laguna, Santa Cruz de Tenerife, Spain
[d] Departamento de Ciencias Médicas Básicas: Unidad de Fisiología, Facultad de Ciencias de la Salud, Universidad de La Laguna (ULL), La Laguna, Santa Cruz de Tenerife, Spain
[e] Sociedad para la Investigación y Asistencia en Salud Mental, Santa Cruz de Tenerife, Spain

ABSTRACT

Introduction. Healthy subjects present higher summer than winter S100B protein concentrations. There is no available information regarding if schizophrenia patients present the same pattern. The aim of this research is to study if patients with schizophrenia present seasonal changes in serum S100B concentrations.

Methods. In fifty-two Caucasian schizophrenia paranoid inpatients meeting DSM-IV criteria, serum S100B protein was measured at 12:00 h and 00:00 h the next day after admission. Patients were recruited for a period of nine months (July-March) and were grouped as summer, autumn or winter group according to the date of admission. Serum S100B levels were measured with an enzyme-linked immunoassay (ELISA) kit.

Results. Patients admitted in winter had significantly higher serum S100B concentrations at 12:00 h and 00:00 h than patients admitted in summer (12:00, winter: 287.5±264.9 *vs.* summer: 33.7±22.6, $p < 0.05$; 00:00, winter: 171.2±143.8 *vs.* summer: 23.3±18.6, $p < 0.05$). Autumn serum S100B concentrations were not significantly different from the summer or winter concentrations (12:00: 128.7±208.8, 00:00: 102.2±153.2). There were no significant differences between 12:00 and 00:00 serum S100B concentrations in any season.

Conclusions. Acutely relapsed paranoid schizophrenia inpatients present significantly higher serum S100B concentrations in winter than summer, the opposite pattern described in healthy subjects, both at midday and midnight. Controlling this seasonal change as source of bias in experimental designs is strongly advisable.

Key words. s100b; schizophrenia; seasonal changes; circadian rhythms; biomarkers

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PACIENTES INGRESADOS POR RECAÍDA AGUDA DE ESQUIZOFRENIA PARANOIDE: CAMBIOS ESTACIONALES, PERO NO CIRCADIANOS, EN LOS NIVELES SÉRICOS DE PROTEÍNA S100B

RESUMEN

Introducción. El objetivo de esta investigación es estudiar si los pacientes esquizofrénicos presentan niveles más altos de proteína S100B en verano que en invierno, como se ha descrito en sujetos sanos.

Método. Se estudiaron 52 pacientes caucásicos que ingresaron por recaída aguda y que cumplían con los criterios DSM-IV de esquizofrenia paranoide. La proteína S100B en suero se midió a las 12:00 y las 00:00 horas el día después del ingreso. Los pacientes fueron reclutados durante nueve meses (julio-marzo) y se agruparon por estación, según la fecha de ingreso, como grupo de verano, otoño o invierno. Los niveles séricos de S100B se midieron con un ELISA.

Resultados. Los pacientes ingresados en invierno presentaron niveles séricos de proteína S100B significativamente más altos a las 12:00 y 00:00 horas que los pacientes ingresados en verano (12:00, invierno: 287,5 ± 264,9 *vs.* verano: 33,7 ± 22,6, $p < 0,05$; 00:00, invierno: 171,2 ± 143,8 *vs.* verano: 23,3 ± 18,6, $p < 0,05$). Las concentraciones séricas

Corresponding author:
Armando L. Morera-Fumero. Departamento de Medicina Interna, Dermatología y Psiquiatría, Facultad de Ciencias de la Salud, Universidad de La Laguna (ULL), 38071, La Laguna, Santa Cruz de Tenerife, Islas Canarias, Spain
E-mail address: amorera@ull.es

de S100B en otoño no fueron significativamente diferentes de las concentraciones de verano o invierno (12:00: 128,7 ± 208,8, 00:00: 102,2 ± 153,2). No hubo diferencias significativas por estación entre las concentraciones diurnas y nocturnas de proteína S100B.

Conclusiones. Los pacientes esquizofrénicos hospitalizados por una descompensación aguda presentan concentraciones séricas de proteína S100B significativamente más altas en invierno que en verano, al contrario de lo descrito en sujetos sanos, tanto a las 12:00 horas como a las 00:00 horas. Al estudiar este biomarcador en la esquizofrenia es recomendable controlar el cambio de estación como fuente de sesgo en los diseños experimentales.

Palabras clave. S100B; esquizofrenia, cambios estacionales; ritmos circadianos; biomarcadores.

INTRODUCTION

Schizophrenia is a disease characterized by delusions, hallucinations, disorganized speech and behaviour, and other symptoms that cause social or occupational dysfunction. Its diagnosis is still based on the clinical interview, so the investigation of biological markers to help with the diagnosis should be a paramount concern[1]. Bioanalysis results rely on the accuracy of laboratory techniques, including aspects of sensitivity and specificity among other parameters. The human subject from whom the biological sample is taken, sometimes, is not considered a source of variability in the biological parameters´ results. Human biomarkers research is a difficult task because some biological variables present values that depend on demographic and individual factors such as gender, ethnic group or chronotype[2,3], among others. High inter-individual and low intra-individual variability have been reported in biological measures[2,4]. Non-individual related variables, such as circadian and seasonal changes[5,6], have also been reported to affect some biological markers.

S100B, a calcium-binding protein that has been proposed as a marker of astrocyte activation and brain dysfunction, has been used as a biological marker in schizophrenia[7]. S100B exerts both autocrine and paracrine functions on neurons and glial cells[8]. In in vitro studies, S100B promotes neural growth and survival at nanomolar concentrations[9] but at micromolar concentrations induces apoptosis[10].

In general, schizophrenia patients present higher S100B levels compared to healthy subjects[11–14]. In healthy subjects, serum S100B do not present circadian changes but present significantly higher summer than winter serum levels[15]. In a previous paper published by our research group we have reported that at admission acute paranoid schizophrenia inpatients present a higher day than night serum S100B levels, but at discharge there was no difference between day and night serum concentrations, matching what happened in the control group[12]. It is not known if S100B protein in schizophrenia patients present seasonal changes as it happens in healthy subjects.

The aim of this research is to explore if patients with schizophrenia present seasonal changes in serum S100B concentrations.

METHODS

Subjects

Fifty-two Caucasian paranoid schizophrenic inpatients meeting DSM-IV criteria participated in the study. For a period of nine months (July 2006-March 2007), acutely relapsed patients were recruited from the psychiatric ward of the University Hospital of the Canary Islands. All patients were independently diagnosed by two clinical psychiatrists. Patients were grouped as summer, autumn or winter season according to the date of admission. Patients with alcohol or substance abuse, physical illness, pregnancy, physical trauma and intellectual disability were excluded. The study protocol was carried out following the Helsinki Declaration and patients or their relatives gave written informed consent before inclusion. The protocol was approved by the Ethics and Investigation Committee of the University Hospital of the Canary Islands.

Psychopathological assessment

Psychopathology was measured with the Positive and Negative Syndrome Scale (PANSS)[16]. The positive, negative and general subscales scores were used as marker of clinical symptoms severity. The PANSS was administered within the 24 hours after admission.

S100B measurement

Blood was collected the day after admission. Samples were collected at 12:00 and 00:00 h. After blood was extracted, samples were placed in vacutainer tubes without anticoagulant and centrifuged at 3000 rpm for 5 minutes, then serum was aliquoted in Eppendorf tubes, and stored frozen at -70º C until analysis. To minimize the assay variance all serum samples were analysed by the technician the same day with the same laboratory batch. The technician was blind with respect to the samples pertaining to summer, autumn or winter season as well as to the moment of the day the sample was collected.

Serum S100B levels were measured with an enzyme-linked immunoassay (ELISA) kit according to the manufacturer instructions (BioVendor, Candler, USA). The BioV-

endor Human S100B ELISA uses a polyclonal anti-cow S100B coated in microtitration wells. The absorbance of the resulting yellow colour product was measured spectrophotometrically at 450 nm in a microplate spectrophotometer reader (Benchmark Plus, Bio-Rad, Hercules, CA, USA). In this ELISA, the lowest detection limit was 17.0 pg./ml. Coefficients of variation were 3.92 % and 5.03 % for intra- and inter-assay variabilities, respectively.

Statistical analyses

Data were analysed using the 21st version of the SPSS statistical package (SPSS, Chicago, Illinois, USA). An analyses of variance (ANOVA) was applied to examine the summer-autumn-winter serum S100B differences. If ANOVA results were significant a post-hoc Bonferroni´s comparison was applied. A paired t-test was applied to compare midday vs midnight serum S100B concentrations. Chi-square was applied to study the association between qualitative variables.

All antipsychotic treatments were transformed into chlorpromazine antipsychotic equivalent doses (CAED)[17] to control the possible effect of the different antipsychotics on S100B concentration.

All statistical tests were two-tailed. Statistical significance level was set at 0.05.

RESULTS

Comparison of demographic and clinical variables according to the season of the patient's admission

The comparison of the demographic and clinical variables according to the season of the patient admission is presented in Table 1 and Table 2. The three seasonal patient samples were similar according to age and gender distribution. Patients admitted in summer, autumn or winter did not differ significantly in the clinical variables.

Comparison of serum S100B concentrations according to the season of the patient's admission

Table 3 presents the result of the ANOVA comparing the serum S100B concentrations by the season of patient´s admission. The comparison of serum S100B concentrations by season elicited a significant effect of season on serum S100B at 12:00 and 00:00 h. Figure 1 represents the post-hoc Bonferroni´s comparison according to the seasons. Serum S100B concentrations were significantly higher in winter than summer both at 12:00 and 00:00 h. Autumn serum S100B concentrations were not significantly different from summer or winter concentrations.

Table 1 Comparison of demographic variables according to the season of the patient's admission

Variables	Summer	Autumn	Winter	F or X^2	P
Age	39.4±9.8	35.2±10.3	36.4±9.1	0.89	0.42
Gender male/ female	12/8	10/4	14/4	4.0	0.13

Table 2 Comparison of clinical variables according to the season of the patient's admission

Variables	Summer	Autumn	Winter	F	P
CAED	673.5± 408.9	795.2± 407.8	797.1± 407.5	0.31	0.74
Positive scale	19.6±4.1	19.2±5.3	21.3±5.4	0.52	0.59
Negative scale	14.9±6.2	13.3 ±5.3	15.4±6.3	0.22	0.81
General scale	24.3±4.9	24.1±3.7	23.7±3.9	0.04	0.96
Age of illness onset	21.5±5.5	24.5±9.2	25.9±6.6	0.86	0.43
Illness duration	16.6±13.0	9.3±7.2	11.7±8.9	1.58	0.22
NPA	4.0±3.2	4.8±3.9	3.0±2.3	0.82	0.45
Body Mass Index	28.9±6.4	26.1±3.2	26.4±4.9	1.20	0.32

CAED: Chlorpromazine Antipsychotic Equivalent Dose; NPA: Number of Previous Admissions

Table 3 Comparison of serum S100B levels according to the season of the patient's admission

Variable	Summer	Autumn	Winter	F	P
S100B 12:00	33,7 ± 22,6	128,9 ± 208,5	287,5 ± 264,9	4,13	0,02
S100B 00:00	23,3 ± 18,6	102,2 ± 153,2	171,2 ±143,8	3,35	0,04

Comparison of serum S100B concentrations according to the blood sampling time of the day by season

The t-test S100B comparison of 12:00 h vs 00:00 h blood sampling by season did not elicit significant differences (Table 4).

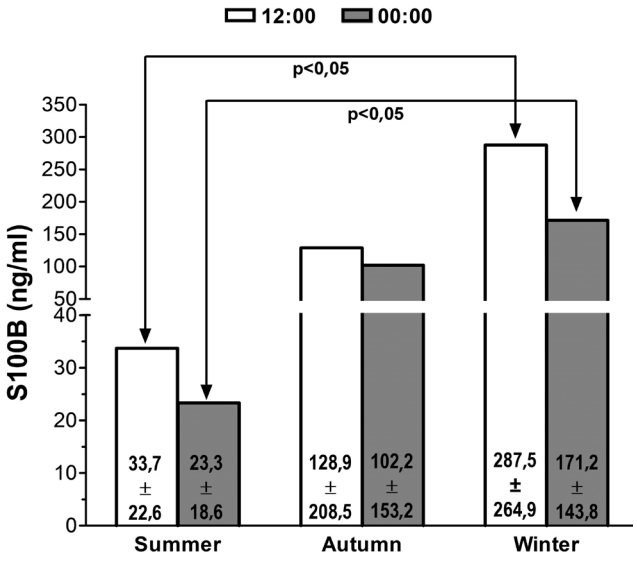

Figure 1 Post-hoc Bonferroni's comparison of serum S100B concentration by season at midday and midnight

Table 4 Comparison of serum S100B levels according to the time of the day in each season

Season	S100B 12:00	S100B 00:00	T	P
Summer	33,7 ± 22,6	23,3 ± 18,6	1,29	0,23
Autumn	128,9 ± 208,5	102,2 ± 153,2	0,77	0,46
Winter	287,5 ± 264,9	171,2 ± 143,8	1,91	0,93

DISCUSSION

To the best of our knowledge, this is the first time that a summer-winter change in the serum concentrations of the S100B protein is reported in acutely relapsed paranoid schizophrenia inpatients. Patients had significantly higher serum S100B concentrations in winter than summer, being the autumn concentrations higher than the summer concentrations but lower than the winter ones. This seasonal pattern, higher in winter than summer, is the opposite pattern reported in healthy subjects, this is, higher in summer than winter[15].

Several medical conditions, such as, heart attack mortality[18], blood pressure in diabetes[19] or psychiatric admissions[20] have a seasonal distribution. Some biological parameters such as melatonin[5], total antioxidant capacity[21] or vitamin D[22] among others, have seasonal changes. In a previous paper we speculated if in healthy subjects the difference in temperature between summer and winter could be a possible explanation for this change[15].

It has been reported that changes in temperature modulate (hyperthermia increases and hypothermia decreases) the permeability of the blood brain-barrier (BBB)[23,24], and therefore a crossing of S100B from the brain to the periphery might account for the increased serum S100B concentration in summer compared to winter. This explanation would not be applicable to our schizophrenia patients because the patients admitted in winter had significantly higher S100B levels than patients admitted in summer.

Hyperpermeability of the BBB has been reported in psychosis[25], but our question would remain unsolved, because the increased permeability of the BBB would explain the increased levels of S100B in schizophrenia subjects compared to healthy subjects, but it would not be a valid explanation for the summer-winter difference of serum S100B concentrations in schizophrenia patients because they presented the opposite pattern.

An alternative and complementary explanation may stem from the study of the genes linked to seasonality[26]. Several genes linked to seasonality have been reported, the ARNTL and NPAS2[27], the CRY1 and CRY2[28] and the PERIOD3[29], among others. Seasonal genes may modulate the expression of the protein production. However, no associations have been described between S100B genes and seasonal genes.

The main strength of our study stems from the fact that the three seasonal samples were similar with respect to the demographic and clinical variables making the results not attributable to those differences.

In our opinion the main limitation of our study is the small size of the sample. It might be possible that the difference between 12:00 and 00:00 h serum S100B concentrations had achieved statistical significance if our seasonal samples had been bigger.

To conclude, acutely relapsed paranoid schizophrenia inpatients present significantly higher serum S100B protein concentrations at 12:00 and 00:00 h in winter than summer, just the opposite result that had been reported in healthy subjects. Autumn S100B concentrations are in the middle between the winter and summer values, not being significantly different from the summer or winter values. Despite that the reason of this alteration is not known, it is strongly advisable to include the season of S100B sampling in the study design in order to control this bias.

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Conflict of interest. The authors declare no conflict of interest.

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