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Evaluation of physiologic pain in relation to pain substances in healthy subjects

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ABSTRACT

This study was aimed at finding a relationship between pain modulators in the blood and physiological pain in apparently healthy Nigerians. It also aimed at establishing a pilot study for finding reference values for plasma levels of substance P, serotonin and tryptophan for the first time among Nigerians. Volunteers were made up of 110 residents of Abuja, aged between 21 and 50 years. Cold pressor test was used to induce pain assessing pain intensity, threshold and tolerance. ELISA was used to assay for plasma substance P, serotonin and tryptophan. Pain parameters from cold pressor test were correlated with plasma pain modulators measured. Results from cold pressor test revealed pain intensity to be 5.79 ± 0.25 cm, pain threshold 28.77 ± 2.32 s and pain tolerance 143.62 ± 24.39 s. Blood plasma level of substance P was 116.52 ± 20.53 pg/mL, serotonin 454.18 ± 30.16 ng/mL and tryptophan 12.77 ± 0.67 μg/mL. There was negative correlation between pain threshold and plasma substance P, pain tolerance and plasma substance P and pain threshold and plasma serotonin. There was however a positive correlation between pain intensity and plasma serotonin. In conclusion, the regression formulas may aid in using cold pressor test to predict blood substance levels of the measured pain modulators in a low resource setting like Nigeria where ELISA test is very expensive.

1. Introduction

Pain is a subjective, psychological experience that teaches us to avoid potentially harmful events, ensures that we protect damaged tissue while it heals, and is essential to promote our longevity. Pain is commonly accepted to be a subjective experience [1], for which the gold standard of measurement is self-report. While self-reported pain provides useful clinical information and proves to be an effective assessment approach in most situations, it can be inadequate or even impossible in certain vulnerable populations. Individuals with major cognitive or communicative impairments, such as unconscious patients or older individuals with dementia, may not be able to provide valid self-reports of pain. For those individuals, there are few methods for determining the presence or absence of pain. While behavioral tools exist (such as those assessing facial expressions, vocalizations, and body movements), they too may fail in individuals with paralyses or other disorders affecting motor behavior [2]. There is, therefore, a need to find objective physiological parameters that may determine how individuals react to pain without recourse to the subjective parameters.

Substance P (SP) is a neuropeptide strongly involved in the process of nociception: primary sensory C-fibre neurons mainly use glutamate as neurotransmitter and SP as co-transmitter [3]. Neurotransmission of SP is negatively modulated by efferent serotonergic neurons (via spinal interneurons). It has been observed that increased levels of SP in brain increases serotonin levels in spinal cord, while serotonin decreases the release of SP into the

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spinal cord [4]. This is a negative feedback loop: high SP levels could lead to a release of its antagonist serotonin. Elevated levels of SP have been found in cerebrospinal fluid of patients with fibromyalgia; but in serum, the concentrations were not found to be altered [5,6]. Since an antagonistic mechanism of serotonin and SP is known in CNS, we investigated the relationship of peripherally measured levels of SP, serotonin and the more stable tryptophan in healthy subjects.

The aim of this study is to establish the pain intensity, pain threshold, and pain tolerance level and also plasma substance P, serotonin and tryptophan level among Nigerians for the first time. This study will also establish a relationship between physiologic pain and the assessed pain modulators in the blood of healthy individuals. Several studies have looked at this relationship in disease states [7–9]. In healthy subjects, pathologic confounders are not there, thus we can see the physiological factors that may alter pain perception and aid in pain management.

Cold pressor test was chosen to assess pain in our subjects considering the fact that it is non-invasive. Also, Nigeria, being a temperate region, most subjects are not adapted to effect of very cold temperature.

2. Materials and methods

2.1. Study subjects

The study area was Abuja, the Federal Capital City of Nigeria. Subjects were drawn from the urban area of Abuja. One hundred and ten subjects between 21 and 50 years comprising of 21 females and 89 males volunteered for the study. Exclusion criteria were: those on any pain medication (whether over-the-counter or prescribed medications), pregnant women and those below 18 years.

All the subjects signed consent form according to Helsinki Declaration of 1964. Ethical approval was obtained from the Ethical Committee of University of Abuja Teaching Hospital, Abuja.

2.2. Cold pressor test

Each participant was seated comfortably on a chair; the non-dominant hand immersed (to about 5 cm above the wrist) in warm water (37 °C) for 3 min. The hand was then removed and immediately submerged in cold water maintained at 2 °C (±0.5 °C) by adding ice cubes and stirred continuously to neutralize the warming effect of participants hands. An insulated water bath with a thermometer was used in both cases. The subject was asked to say "Pain" on the first experience of pain sensation (pain threshold) but still left his/her hand in the cold water until the pain became unbearable (pain tolerance), at that point he/she withdrew the hand from the water. Both times were recorded. However if the subject did not withdraw the hand after 5 min in cold water, the investigator stopped the test to prevent injury [10]. The subjects were then given the visual analogue scale to record the pain intensity (Fig. 1).

2.3. Procedure for blood sample collection

A cannula was inserted into the subjects' antecubital veins on the dominant side. All subjects were rested supine for at least 10 min before blood sampling. The blood samples were taken between 9:00 am and 12:00 noon daily. The blood (5 mL) was collected into cooled tubes prepared with EDTA. The tubes were transported on ice and centrifuged at 1000 × *g* for 15 min within 30 min of collection. Plasma was aliquoted into plain polypropylene tubes and stored at ≤−20 °C until it was ready for use. Plasma substance P, serotonin and tryptophan were estimated by ELISA. The procedures were as described by the manufacturer of the kit (Abnova Co., Taipei, Taiwan). All reagents were warmed to room temperature for at least 30 min prior to opening. All standards and samples were run in duplicate.

2.4. Estimation of plasma substance P

50 μL of standard diluent (assay buffer) was pipetted into the NSB and the Bo (0 pg/mL standard) wells. 50 μL of standards #1 through #6 was added into the appropriate wells. 50 μL of the samples was then added into the appropriate wells. 50 μL of assay buffer was added into the NSB wells. 50 μL of blue conjugate was then added into each well, except the total activity (TA) and blank wells. 50 μL of yellow antibody was pipetted into each well, except the blank, TA and NSB wells. It was then left to incubate at room temperature on a plate shaker for 2 h at ∼500 rpm. The plate was covered with the plate sealer. Then the contents of the wells were emptied and washed by adding 400 μL of wash solution to every well. This was done three times. After the final wash, the wells were emptied. Then 5 μL of the blue conjugate was added to the TA wells, and 200 μL of the p-Npp substrate solution to every well. It was left to incubate at room temperature for 1 h without shaking. Then 50 μL of stop solution was added to every well. This stopped the reaction and the absorbance was read at 450 nm using Spectrophotometer (Accurex AT-112®).

2.5. Estimation of plasma serotonin

25 μL of standards, 25 μL of controls, and 25 μL of plasma was pipetted into the respective reaction tubes. Then we added 500 μL of acylation buffer to all tubes, and 25 μL of acylation reagent to all tubes and mixed thoroughly and incubated for 15 min at room temperature (20–25 °C). 25 μL of the acylated standards, controls and samples were pipetted into the appropriate wells of the serotonin microtiter strips. 100 μL of the serotonin antiserum was pipetted into all the wells and then incubated for 30 min at room temperature on a shaker (∼600 rpm). The contents of the wells were discarded and washed 3 times thoroughly with 300 μL of wash buffer and dried by tapping the inverted plate on absorbent material. 100 μL of the conjugate was then pipetted into all the wells and incubate for 15 min at room temperature on a shaker (∼600 rpm). The contents of the wells were discarded and washed 3 times thoroughly with 300 μL of wash buffer and dried by tapping the inverted plate on absorbent material. 100 μL of the substrate was then pipetted into all wells and incubate for 15 ± 2 min at room temperature on a shaker (∼600 rpm). 100 μL of the stop solution was added to each well and the microtiter plate shaken to ensure a homogeneous distribution of the solution. The absorbance of the solution in the wells was read within 10 min, using a microplate reader (Spectrophotometer (Accurex AT-112®) set to 450 nm and a reference wavelength between 620 nm and 650 nm.

The calibration curve was obtained by plotting the absorbance readings (calculated mean absorbance) of the standards (linear, *y*-axis) against the corresponding standard concentrations (logarithmic, *x*-axis). Then non-linear regression was used for curve fitting. The concentrations of the samples and controls were read directly from the standard curve.

2.6. Estimation of plasma tryptophan

25 μL of the prepared standards, controls and samples was pipetted into the appropriate wells of the tryptophan microtiter strips. 50 μL of the tryptophan antiserum was pipetted into all wells and mixed shortly. It was then covered with adhesive foil and incubated for 15–20 h (overnight) at 2–8 °C. After that the contents of the wells was discarded and washed 3 times thoroughly with 300 μL of wash buffer. It was then dried by tapping