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Objective clinical pain analysis using serum cyclooxygenase-2 and inducible nitric oxide synthase in American patients

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ABSTRACT

Background: Pain is a multidimensional condition of multiple origins. Determining both intensity and underlying cause are critical for effective management. Utilization of painkillers does not follow any guidelines relying on biomarkers, which effectively eliminates objective treatment. The aim of this study was to evaluate the use of serum cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) as pain biomarkers. This work could significantly advance the diagnosis and treatment of pain.

Methods: We assessed the potential utility of serum COX-2 and iNOS as objective measures of pain in a sample of American patients. Pain was scaled between level 0–5 in accordance with the level reported by the patients. Blood samples were collected from 102 patients in the emergency room. Sandwich ELISA was used to determine the COX-2 and iNOS levels in the blood serum while statistical analysis was performed using Pearson product-moment correlation coefficients, Regression and Receiver Operating Characteristics (ROC) analyses. The biomarker results were also compared with self-reports of pain by the patients using conventional pain ratings and patients were asked to report the cause of the pain. Pain levels were clustered into four groups as 0 [self-reported 0], 1 [self-reported as 1], 2 [self-reported as 2 and 3] and 3 [self-reported as 4 and 5]. Co-expression of COX-2 and iNOS could significantly alter pain development and its sensitization. Therefore, iNOS dependent COX-2 levels were employed as categorized level.

Results: Self-reported pain levels did not show a correlation with the serum level of COX-2 and iNOS. The lack of correlation is attributed to multiple reasons including patients' intake of painkillers prior to participation, painkiller intake habit, chronic diseases, and subjectivity of self-reported pain. Increased serum COX-2 levels were reported in relation to the subtypes of these health issues. Further, 83% of the patients who reported pain also showed the presence of COX-2 in serum, while only 53% of the patients showed the presence of iNOS in serum. Moderate relation was found between the clustered pain level and categorized COX-2 and iNOS- levels.

Conclusions: The findings support the requirement of further studies to use COX-2 and iNOS as prognostic biomarkers for objective quantification of pain at the clinical level.

1. Introduction

Intracellular enzymes/proteins in serum can provide a clear indication of severity, mechanism, and prognosis of ongoing or upcoming pathologic conditions [1]. Release of these protein biomolecules into blood circulation can result from multiple pathologies, which could complicate their interpretation [2]. Therefore, a set of biomarkers have been utilized in the characterization of diseases with their status in clinics worldwide [3]. From this perspective, pain, a highly complex noxious signal resulting from a variety of pathologic or even non-pathologic conditions [4], requires a specialized set of biomarkers to objectively provide a greater understanding of the underlying reason,

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along with an assessment of severity [5]. However, none of the health authority institutions worldwide has approved any biomarkers for objective pain characterization [6].

The 2 most targeted pain-related intracellular enzymes, namely cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS/NOS2), were studied to evaluate their potential for "biomarker set" development efforts. COX-2, in most tissues, is the inducible form of cyclooxygenases, whose characteristic overexpression in relation to pain has been reported as an element linked to both the specifics of the pathologic condition and to severity [7]. Drug administration to block COX-2 activity has been shown in clinical studies to suppress pain and post-surgical pain development resulting from a wide-range of procedures, including dental [8], knee [9], oral [10] and abdominal surgeries [11] or acute pains including dental [12], throat [13] and non-classified acute sources [14]. There are only limited biopsy based studies examining COX-2 levels in relation to pain, which is not an effective approach for routine biomarker monitoring analysis [15]. Similar to COX-2, iNOS/NOS2 is the inducible version of nitric oxide synthases for most tissues, which plays a dual role in the development and sensitization of pain [16] and pathologic conditions, such as inflammation and infection through its nitric-oxide synthesis capability [17]. Despite the fact that these two enzymes are among the most widely targeted enzymes [16, 18], research on their potential as pain biomarkers in circulating blood has not been fully explored.

2. Design and methods

The data collection protocol was approved by the Institutional Review Boards (IRB) of Binghamton University, SUNY Upstate Medical University, and United Health Services (UHS) Hospitals at Binghamton. Informed consent was obtained from each subject and they have not been identified except only by number, not name or initials. Patients ≥18 y, admitted through the emergency room (ER) of the UHS Wilson Medical Center at Johnson City, NY between March and October of 2016, were recruited for data collection for this study. The rationale of recruiting the patients from ER room was to obtain heterogenous patient profile in order to evaluate serum COX-2 and iNOS levels in response to different types of pain conditions. The patients, who came in day time on weekdays, were informed about the study by the nurses. None of the patients was under psychological stress and out of conscious control. The ones who accepted to participate in the study from the researchers were directed to the research team. Then, the study was first briefly explained to the patients by a member of our research team. If the patient was willingly to participate in the study, the researcher gave the informed consent form to the patient. Patients received a modest compensation ($30.00) for their participation in the study. Upon receiving the signed consent form from the patient, the researcher explained the concept of the study and interviewed the patients with the survey given in Supplemental Table 1. During this survey, patients were asked to rate their level of pain on a scale of 0–5; 0 = no pain, 1 = feeling pain but not disturbing, 2 = feeling pain and little disturbing, 3 = strong pain and requires painkiller usage, 4 = very strong pain and distracting from working and requires urgent pain-killer usage, and 5 = unbearable pain requiring urgent pain-killer usage and rest as well as causing anxiety. Patients were also asked to report the cause of the pain. Additionally, patients were asked about their age, smoking and drinking habits, and any medications taken on a regular basis, or taken specifically for the pain (either prior to arriving at the ER, or given to them by the ER staff). After the survey, another member of the research team from the Computer Science Department of Binghamton University made a video-recording of the patients. Following video recording, the on-staff phlebotomist or nurse withdrew a small amount of blood from the patient (about 4 cm^3). Blood was stored in a cooled container by the experimenter, and returned to the lab for processing each evening.

Data were collected from 115 patients in the ER room, of which 50% were males and 50% were females. The female patients were between 19 and 77 y (average 48.3) while the male patients were between 24 and 77 y (average 48.7 y). The reported causes of pain by the 102 patients (whose blood samples were processed) were unknown (n = 21), inflammation (n = 15) and accident (n = 12) as single-source causes, and polyetiological (n = 54) for which mostly accident, abdominal, kidney, chest, back pain and inflammation/cancer related pains were reported.

Levels of COX-2 and iNOS were evaluated with sandwich ELISA as detailed elsewhere [19,20]. Briefly, blood samples in red-black tap BD tubes were centrifuged at 2000 rpm for 15 min at room temperature. The serum was then placed into −20 °C storage until used in the ELISA test. None of the sample was stored for > 1 month. The frozen samples were thawed at 37 °C for 10 min, and then centrifuged at 3000 rpm for 5 min. The samples were then 3 times-diluted in pH 7.6 PBS buffer, and 100 μL of samples as 3-replicates were used in the ELISA studies. ELISA studies were performed by members of the team from the Department of Chemistry of Binghamton University.

2.1. Recording pain level, and correlating pain level with serum COX-2 and iNOS levels

Three prominent factors were taken into account in classification of the self-reported pain level. (i) Level of pain before taking a pain-killer was recorded as the presenting level of pain. (ii) Increased pain level with motion was not accepted as the recorded level of pain; (iii) the level of pain at rest was used as the recorded pain level.

Interpretation of the relation between the level of pain and serum COX-2 and iNOS levels, or iNOS-dependent COX-2 levels was guided by four criteria. (i) The level of pain was individually correlated with level of COX-2 and iNOS. (ii) If the patient reported more than one reason for the pain, the patient was evaluated for each type of reported pain. (iii) iNOS-dependent COX-2 levels in relation to the level of pain were interpreted to enlighten their cooperative effect. (iv) The pain classifications were simplified as unknown (n = 21), inflammatory (n = 15), accident (n = 12) and polyetiological (n = 54) (Fig. 1). Polyetiological pain reasons were overwhelmingly related to "abdominal pain", "kidney related pain", "chest pain" and "back pain".

2.2. Ranging serum COX-2 and iNOS levels

Due to the fact that biomarkers at slightly different levels could be a sign of similar pathological conditions, serum COX-2 and iNOS levels were categorized into 3 groups based on median, 3rd quartile and mean in order to seek the correlation with the level of pain. It is well-known that iNOS could worsen the COX-2 mediated pathogenesis through a variety of mechanisms including COX-2 overexpression, post-translational modification and peroxinitrite formation. Here it is noteworthy to mention that co-expression of COX-2 and iNOS greatly alter pain development and its sensitization [16]. Therefore, iNOS dependent COX-2 levels were categorized to seek the correlation as well.

Data analysis and classifications were conducted based on the obtained survey using Pearson product-moment correlation coefficients (r) showing the relationship between 2 variables, similar to the classification described elsewhere [21]. These relationships can be ranked: 0 refers to no relation at all, between 0 and ± 0.2 refers to negligible; between ± 0.21 and ± 0.35 refers to weak, between ± 0.36 and ± 0.67 refers to moderate, between ± 0.68 and ± 0.9 refers to strong and between ± 0.91 and ± 1 refers to very strong [21]. Besides, regression analysis was also performed to evaluate the relation between the level of pain and serum COX-2 and iNOS levels. Finally, ROC curve analysis was performed based on standard procedures. When a significant cut-off value was observed, the sensitivity, specificity, area under curve (AUC), standard error, p-value and confidence interval (CI) were presented. While evaluating the area under the curve, a 5% type-I error level was used to accept a statistically-significant predictive value of the