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Circadian time structure of circulating plasma lipid peroxides, antioxidant enzymes and other small molecules in peptic ulcers

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ABSTRACT

Background: The circadian rhythm, as part of a broad time structure (chronome) of lipid peroxides and antioxidant defense mechanisms may relate to prevention, efficacy and management of preventive and curative chronotherapy.
Methods: Fifty newly diagnosed patients with peptic ulcers, 30–45 years of age, and 60 age-matched clinically healthy volunteers were synchronized for one week with diurnal activity from about 06:00 to about 22:00 and nocturnal rest. Breakfast was served around 08:30, lunch around 13:30 and dinner around 20:30. Drugs known to affect the free-radical systems were not taken. Blood samples were collected at 6-hour intervals for 24 h under standardized, presumably 24-hour synchronized conditions. Plasma lipid peroxides, in the form of malondialdehyde (MDA), blood superoxide dismutase (SOD), glutathione peroxide (GPx), glutathione reductase (GR), catalase (CAT) activities, and serum total protein, albumin, ascorbic acid, total serum cholesterol, and HDL-cholesterol concentrations were determined.
Results: By population-mean cosinor analysis, a marked circadian variation was demonstrated for all variables in healthy subjects and in ulcer patients ($p < 0.001$). As compared to controls, patients had a lower MESOR of MDA, SOD, GPx, GR, ascorbic acid, and HDL-C. They also had smaller circadian amplitude of SOD, CAT, GPx, GR, ascorbic acid, T-C, and HDL-C, but larger circadian amplitude of MDA and albumin. As compared to healthy subjects, the circadian acrophase of ulcer patients occurred later for MDA and GR and earlier for GPx.
Conclusion: Mapping circadian rhythms, important chronome components that include trends with age and extra-circadian components characterizing antioxidants and pro-oxidants, is needed for exploring their putative role as markers in the treatment and management of peptic ulcers.

1. Introduction

Chronobiology defines and quantifies biological rhythms as parts of a broader temporal organization of life and of its environment as chronomes, at every level of structural and functional complexity. It focuses on the relative contributions of exogenous and endogenous periodic phenomena in so far as they can be separated in systems open to their environment. The effect of time can thus be considered as a systemic and to a certain degree, predictive factor in the quantitative assessment of biological data [1]. Acute injuries of gastric mucosa are increasing in frequency. Patients with alcohol and drug-induced injury, trauma, hemorrhage, and sepsis may survive their initial injury because of modern therapeutic advances only to succumb later to bleeding from their gastric mucosa [2]. Acute injuries of the gastric mucosa continue to be a subject of research interest, not only because of therapeutic challenge and many unsolved problems related to their etiology, but because they are quite common and life-threatening. The stomach is an organ in direct contact with external pathogens; by presenting a strong acid environment, it has a special biological defense mechanism that eliminates such pathogens. However, *Helicobacter pylori* manage to live in the stomach by breaking through this defense line. In response to the colonization of this bacterium, gastric mucosa can be exposed to severe oxidative stress with considerable inflammatory cell accumulation, which might be related to the development of gastric mucosal as well as neuromuscular disorders. Oxidative stress has been reported to be one of the major contributors to the development of stomach diseases. Recent therapeutic options such as gastro-protective agents including antioxidant properties (i. e., rebamipide) can modulate the

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level of oxidative stress to enhance anti-inflammatory or antioxidant capacity [3]. The involvement of free-radicals in the pathogenesis of peptic ulcers and gastric carcinoma has been well defined in experimental animals [4]. Oxidative stress relates to a variety of conditions that stimulate either additional ROS production or a decline in antioxidant defenses. Oxidative stress is not only involved in the pathogenesis of gastric inflammation, ulcerogenesis, and carcinogenesis in *H. pylori* infection, but also in life-related diseases including atherosclerosis, hypertension, diabetes mellitus, ischemic heart disease, cirrhosis of liver and malignancies [5]. Circadian variations of different blood and urinary variables have been reported in health and disease in Indian populations [6–12]. Similarly, chronomics of circulating lipid peroxides, antioxidant enzymes and other small molecules have been reported in pulmonary tuberculosis, malignancies and cirrhosis of the liver [13–15].

Apart from our own investigations [14,15], many other studies reported on circadian rhythmicity of oxidative stress markers in health as well as disease [16–20]. Information is lacking, however, regarding the circadian rhythms of circulating lipid peroxides and intracellular antioxidant enzymes as they may be altered in the hemolysate of patients with peptic ulcers. The present study aims to fill this gap by providing reference values for circadian changes of studied profiles in clinical health. Any deviation from such norms in peptic ulcers is assessed in an attempt to understand the mechanism of oxidative stress and its involvement in ulcerogenesis. A better understanding of the role of oxidants and antioxidants may thus be gained for preventing and/or minimizing free-radical damage associated with peptic ulcer.

2. Patients and methods

This investigation includes 50 newly diagnosed endoscopically-validated cases of peptic ulcers admitted in the Medical Wards of Gandhi Memorial and Associated Hospitals, King George's Medical University, Lucknow, and 60 clinically healthy volunteers, comprised mainly of medical students, staff members and their families. The age of the healthy volunteers ranged from 21 to 45 years and that of the patients from 30 to 45 years. This study was approved by the Institutional Ethics Committee. The patients were thoroughly examined to ensure the absence of any other disease known to alter the free-radical system. Patients already on antioxidants, such as vitamin E or C, or captopril (containing an SH group) were excluded from the present study. Prior to the collection of blood samples, participants refrained from taking any drug preparation that would affect or alter the oxidative stress or its defense mechanism. All participants were synchronized for 1 week to a schedule of diurnal activity from about 06:00 to about 22:00 and nocturnal rest. All subjects took their usual (although not identical) meals three times daily; breakfast around 08:30, lunch around 13:30 and dinner around 20:30, without any change in their fluid intake. At 06:00, 12:00, 18:00 and 00:00, 6 ml of blood was collected from each subject in plain and sterile vials containing heparin as anticoagulant. The plasma was separated and analyzed for lipid peroxidation in terms of malondialdehyde (MDA) [21]. The hemolysate was prepared from the red cells and used for the measurement of the activities of the following enzymes: superoxide dismutase (SOD) [22], catalase [23], glutathione peroxidase (GPx) [24], and glutathione reductase (GR) [25]. Serum total protein, albumin, total cholesterol (T-C), high-density cholesterol (HDL-C) and ascorbic acid were measured spectrophotometrically [26–28].

Data were evaluated by conventional statistical analyses and by the single and population-mean-cosinor procedures [29–31]. Accordingly, the MESOR (a chronome-adjusted mean), the circadian double amplitude (a measure of the extent of predictable change within a day) and the circadian acrophase (a measure of the timing of overall high values recurring each day) were estimated. Circadian rhythm parameters estimated by population-mean cosinor were compared between the ulcer patients and the healthy controls by parameter tests [30].

3. Results

Results are summarized in Tables 1 and 2. A circadian rhythm was invariably demonstrated by population-mean cosinor ($p < 0.001$) and 1-way analysis of variance ($p < 0.001$, except for SOD of patients for which $p = 0.0180$. separately in healthy volunteers and patients with peptic ulcers for all studied variables. By comparison to the healthy controls, the patients with peptic ulcers had a lower MESOR of MDA ($F = 8.589$; $p = 0.004$), a larger circadian amplitude ($F = 21.977$; $p < 0.001$) and a later circadian acrophase (17:16 vs. 16:20; $F = 5.513$; $p = 0.021$). The MESOR of SOD activity was lower in patients ($F = 47.201$; $p < 0.001$) and the circadian double amplitude was smaller (0.93 vs. 2.85 Units/ml RBC; $F = 48.537$, $p < 0.001$). The circadian double amplitude of CAT activity was smaller in the patients (0.99 vs. 2.55 Units/ml RBC; $F = 77.488$, $p < 0.001$). GPx activity was lower at all sampling times in ulcer patients by comparison with healthy subjects, with a lower MESOR ($F = 45.139$, $p < 0.001$) and a smaller circadian amplitude ($F = 50.034$, $p < 0.001$), the circadian acrophase occurring earlier (13:56 vs. 16:16; $F = 15.768$, $p < 0.001$). GR activity was lower at all sampling times in ulcer patients by comparison with healthy controls. Their MESOR of GR activity was lower ($F = 81.049$, $p < 0.001$) and their circadian amplitude smaller ($F = 122.464$, $p < 0.001$), and with a circadian acrophase occurring 2 h later ($F = 15.358$, $p < 0.001$). No difference was found for total serum protein between ulcer patients and healthy subjects. The patients had a larger circadian amplitude of serum albumin ($F = 7.478$, $p = 0.007$). The circadian amplitude of T-C was smaller in ulcer patients ($F = 11.528$, $p = 0.001$). The circadian amplitude of HDL-C was also smaller in patients ($F = 7.607$, $p = 0.007$) who had a lower MESOR ($F = 32.020$, $p < 0.001$). In comparison to healthy participants, ulcer patients had a lower MESOR of serum ascorbic acid ($F = 24.580$, $p < 0.001$) and a smaller circadian amplitude ($F = 23.709$, $p < 0.001$).

4. Discussion

We found a marked circadian variation in MDA, total protein, albumin, total cholesterol, HDL-C and ascorbic acid concentration and SOD, CAT, GPx and GR activities in healthy Indians and patients suffering from peptic ulcer disease. Contrary to other reports [3–5,32–34], we found plasma lipid peroxides to be lower in patients with peptic ulcer. We also found ulcer patients to have a delayed circadian acrophase, a result not previously reported to our knowledge. Similarly, there is apparently no report in the available literature regarding the circadian nature of lipid peroxidation in ulcer patients. The decreased concentration of plasma MDA may bring about some imbalance between protective and aggressive factors at the tissue and cellular levels, leading to mucosal erosion and ulceration in such patients. Lower plasma lipid peroxide concentrations in such patients may be due to participation and/or association of reactive oxygen species in tissue injury and thus, ulceration. Lipid peroxidation in cell membranes and subcellular organelles has been proposed as a primary mechanism for cellular membrane dysfunction and tissue injury associated with free-radical-initiated processes. Lower concentrations of lipid peroxides may disturb relations between protective and aggressive factors at the tissue and molecular levels, leading to oxidative stress-related gastric mucosal injury. Although much is known about the chemistry of lipid peroxidation and cellular defense mechanisms, chronobiological studies are needed to quantify the various cellular components involved in these processes to achieve better efficacy and safety of the novel therapies in the management, prognosis and treatment of the disease. Circadian rhythms of pro-oxidants have been mapped to explore their putative chronotherapeutic role as markers in the prevention and management of different types of malignancies, pulmonary tuberculosis and cirrhosis of the liver [13–15]. Oxidative stress is one of the major contributors for the development of gastric organic disorders. Recent therapeutic gastroprotective agents can modulate the level of oxidative stress to

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