Inflammatory Bowel Diseases (Salivary Biomarkers)

Subjects: Pathology

Contributor: Kacper Nijakowski

Saliva as a biological fluid has a remarkable potential in the non-invasive diagnostics of several systemic disorders. Inflammatory bowel diseases are chronic inflammatory disorders of the gastrointestinal tract.

Keywords: inflammatory bowel disease ; saliva ; biomarkers

1. Introduction

Saliva is an opalescent biological fluid containing a mixture of secretions from three pairs of major (including parotid, submandibular and sublingual) and many minor salivary glands, as well as gingival crevicular fluid. It performs numerous functions: protective, digestive, defensive and diagnostic. Saliva, like blood, contains numerous enzymes, hormones or antibodies [1]. Thanks to the new techniques, such as molecular diagnostics or nanotechnology, the small volume of fluid and low concentrations of examined elements are no longer an obstacle [2]. Saliva is collected easily and non-invasively, eliminating the stress caused to patients during blood sampling. Saliva collection is safe even for less qualified medical personnel and does not require – like clotting blood – special conditions before delivery to the laboratory [3]. New future molecular technologies focus on the analysis of proteins and nucleic acids contained in saliva [4][5][6]. Promising future solutions are lab-on-a-chip (LOC) and point-of-care (POC) technologies, which would be portable, miniaturised devices giving immediate results, based on the principles of conventional ELISA tests or nucleic acid hybridisation [7]. Saliva has an impressive potential in the diagnostics of several systemic disorders, including oncological, cardiovascular, endocrine, autoimmune, neurological, infectious or just gastrointestinal diseases [8].

Moreover, it should be emphasized that oral inflammation may change the composition of saliva and interfere with the expression of selected diagnostic proteins. The common oral diseases include dental caries and periodontal diseases. Vitorino et al. [9] identified higher concentrations of amylase, immunoglobulin A, lactoferrin, lipocalin, cystatins and proline rich acid proteins (PRPs) in caries-free patients. Rudney et al. [10] indicated that elevated levels of staterin and truncated cystatin S may be associated with an increased risk of caries development. Furthermore, Fábián et al. [11] reviewed salivary proteins linked with periodontitis. These include immunoglobulins, heat shock protein Hsp70, cystatin S, amylase, calprotectin, histatin, lysozyme, lactoferrin, defensin, peroxidase, PRPs and mucins. Both active and passive smoking influence the condition of periodontium. In active smokers, Kibayashi et al. [12] observed significantly decreased concentrations of prostaglandin E2, lactoferrin, albumin, aspartame aminotransferase, lactose dehydrogenase and alkaline phosphatase. In passive smokers, Nishida et al. [13] determined reduced levels of interleukin 1β, albumin and aspartame aminotransferase.

Inflammatory bowel diseases (IBD) comprise chronic inflammatory disorders of the gastrointestinal tract, affecting millions worldwide [14]. Despite many investigations, the exact etiopathogenesis remains unknown. Potential factors involve genetic predisposition, environmental conditions and immunological dysfunctions. The main forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Although transmural inflammation in CD may affect any part of the gastrointestinal tract from the oral cavity to the rectum, it occurs most frequently in the terminal ileum or the large intestine. In contrast, UC usually concerns only the large intestine and is limited to the mucosal layer [15]. The active IBD may manifest with symptoms, such as chronic diarrhoea, abdominal pain, weight loss, fever or even severe complications, e.g., intestinal fistulas or intra-abdominal abscess [16]. The diagnosis is confirmed based on the endoscopy and histological examination of the inflamed mucous membrane biopsy (current gold standard). In IBD patients, it may occur oral lesions – specific (e.g., for CD cobblestoning, mucosal tags or deep linear ulcerations, and for UC pyostomatitis vegetans) or nonspecific, such as aphthous stomatitis, angular cheilitis or atrophic glossitis [17].

2. Oxidative Status Markers

Oxidative stress reflects the imbalance between excessively produced reactive oxygen species (ROS) and the insufficient activity of antioxidants which may lead to cell and tissue damage. Among the most important salivary antioxidants, the following should be mentioned—uric acid, albumin and transferrin. They play a key role by scavenging free oxygen radicals (e.g., peroxyl or hydroxyl) or binding to ions such as iron and copper which promote oxidative damage [18]. Furthermore, the main intracellular antioxidants are superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) [19][20]. Nitric oxide (NO), a free radical messenger molecule, is produced via the action of nitric oxide synthase on the L-arginine. Low levels of nitric oxide are thought to be physiological and protective, whereas its high levels as proinflammatory and injurious [21]. Recently, in CD patients, the overexpression of ROS and NO, as well as lower concentrations of numerous antioxidants, have been studied in intestinal mucosa or blood but not in saliva [20][21][22][23][24][25][26][27][28].

The study by Jahanshahi et al. [29] was the first evaluation of salivary oxidative and nitrosative stress in IBD patients. The antioxidant capacity of saliva was determined by measuring its ability to reduce Fe^{3+} to Fe^{2+} (FRAP—ferric reducing antioxidant power). Lipid peroxidation in samples was assessed based on the concentrations of adducts containing malondialdehyde (MDA) with 2-thiobarbituric acid (TBA). Levels of MDA, as the end product of the oxidation of polyunsaturated fatty acids, allow establishing the lipid peroxidation extent; while NO was assayed on the basis of the enzymatic conversion of nitrate to nitrite by nitrate reductase. Antioxidant power in the saliva of CD patients was significantly lower compared to healthy subjects. In addition, salivary concentrations of MDA increased significantly in this group. In contrast, salivary levels of FRAP and the products of lipid peroxidation were normal in UC patients. Explaining these surprising findings, the authors proposed that UC may not affect salivary glands and buccal mucosa. In CD several organs, including salivary glands, are oxidatively stressed as a result of chronic inflammation. However, the analysis of nitrosative stress showed a significant increment of NO in the saliva of both CD and UC patients in comparison to the control group. It is the first study determining increased salivary NO levels in IBD patients and supporting the theory that NO production may be an etiologic factor of these diseases as could cause epithelial and mucosal injuries. Moreover, researchers determined the salivary concentrations of epidermal growth factor (EGF). This main member of the EGF family is produced by submandibular glands and Brunner's glands in the duodenum [30]. EGF stimulates the proliferation of epithelial and nonepithelial cells present in the gastrointestinal tract. It is also a stimulator of the expression of brush border enzymes and intestinal electrolyte and nutrient transport in enterocytes, as well as a promotor of intestinal healing [31][32]. In this study, CD patients showed compensated high concentrations of EGF and UC patients not depleted. The reason for these findings is unclear; the authors suggested that the altered levels of other EGF-family peptides, sharing the same receptor EGFR, may interfere.

In CD patients, the salivary levels of FRAP, uric acid and albumin were significantly reduced, as well as salivary transferrin and thiol groups but not significantly. MDA and NO concentrations were increased, respectively, five- and four-fold in comparison to the healthy subjects. No relationship was found between oxidative stress markers and NO. The disease activity was assessed according to Crohn's Disease Activity Index (CDAI), including the number of liquid stools, the severity of abdominal pain, general well-being, extraintestinal manifestations (e.g., arthralgia, aphthous ulcers, anal fissures, fistulae or abscesses), abdominal mass, use of antidiarrheal drugs, haematocrit and body mass. CDAI significantly correlated with MDA, FRAP and the interaction between them (r = 0.8, p < 0.00005). In the constructed model, patients with a normal or low total antioxidant capacity had the lowest and the highest disease activity, depending on the MDA concentrations, whereas patients with high FRAP and high MDA levels had moderate disease activity due to the active defence of the organism against oxygen radicals. In turn, UC patients demonstrated an approximately four-fold increase in NO levels, without dependence on the activity of the disease. This finding was conflicting with previous studies concerning UC severity and NO levels in regions different to the oral cavity. The authors found that salivary oxidative stress could play a role in the pathogenesis of the CD (but not UC), modifying its course, and recommended further investigations. The results on salivary TGF-β1 from both studies will be discussed in the next section about inflammatory markers.

Serum and salivary MDA concentrations were increased, and the GSH levels decreased in active CD patients compared to inactive CD patients and controls. Levels of MDA in the saliva were higher than in the serum. Salivary FRAP levels were lower in CD patients, but not significantly, and there were no differences depending on the disease activity. The CAT activity was reduced in active CD patients in comparison to other groups, while in the earlier investigation, the authors reported a similar tendency for the SOD activity [33]. In the present study, a strong positive correlation between the serum or salivary MDA concentrations and CDAI values (both r = 0.8, p < 0.001) was observed. In addition, negative correlations between FRAP or GSH levels in the saliva and CDAI values were detected (r = -0.4, p = 0.04 and r = -0.05, p = 0.01, respectively). Moreover, there were significant positive correlations between the serum or salivary MDA concentrations

with the CRP level (r = 0.6, p < 0.001 and r = 0.7, p < 0.001, respectively) and the platelet count (r = 0.6, p < 0.001 and r = 0.6, p = 0.001, respectively), as well as the negative with the haemoglobin level (r = -0.6, p < 0.001 and r = -0.05, p = 0.01, respectively). These relationships were determined using the Spearman rank correlation coefficient. The above significant associations between the increased MDA levels and clinical symptoms of disease severity suggested that it could have a valuable indicator for early CD diagnosis. The diagnostic usefulness of oxidative stress indicators was calculated by receiver operating characteristic (ROC) curves. Currently, the CRP seems to be the best biochemical marker for assessing and monitoring CD activity; however, more useful than for its diagnosis. The ROC analysis showed the good utility of MDA and CRP in differentiating active from inactive CD according to CDAI values (AUC = 0.95, cut-off point = 3.82 and AUC=0.85, cut-off point = 4.25, respectively). The authors proposed that salivary biomarkers, such as MDA, can be used to assess oxidative stress in CD patients, both with the active course and the clinical remission.

3. Inflammatory Cytokines

However, the salivary biomarkers of inflammatory processes in IBD patients have been rarely investigated. The limitation may be the fact that the IBD patients are chronically treated with anti-inflammatory drugs (such as aminosalicylates, thiopurines or glucocorticoids), affecting the measured cytokines.

Salivary IL-6 concentrations were significantly higher in CD patients but not significantly in UC patients. In contrast, a significant difference in plasma IL-6 levels was observed in both groups (p < 0.001). In UC patients, significant positive correlations between salivary IL-6 concentrations and plasma concentrations or AI score (r^2 = 0.81, p < 0.01 and r^2 = 0.61, p < 0.05, respectively) were demonstrated, as well as negative with albumin level (r^2 = 0.83, p < 0.01). No similar relationships were found in the CD patients. IL-6 levels in the saliva of both groups were not associated with CRP levels.

The salivary flow rate did not differ between these groups or correlate with the prevalence of oral manifestations in both CD groups. The ELISA analysis demonstrated significantly elevated salivary levels of IL-1β, IL-6 and TNF-α in active CD patients. No significant differences were found between inactive CD patients and controls. In the Spearman rank test, positive correlations between higher salivary concentrations of IL-6 or TNF-α and the prevalence of specific oral lesions in active CD were observed, while no such association was seen for IL-1β. The researchers speculated that the reason for this finding might be the antagonistic influence of CD drugs on salivary IL-1β. In addition, the present study showed a relationship between oral manifestations and standard laboratory parameters (such as RBC, haemoglobin, platelets, CRP), however, not with CDAI values. Authors concluded that active CD patients had altered cytokine production reflected by increased concentrations of proinflammatory cytokines in the saliva. Salivary cytokine levels may be sensitive biomarkers of CD activity.

TGF-β1 plays an essential anti-inflammatory role by counteracting TNF-α [34][35][36]. As mentioned earlier, Rezaie et al. [37][38] investigated TGF-β1 levels in IBD patients. Although TGF-β1 concentrations were significantly increased in CD patients compared to control subjects, they had no correlation with CDAI or oxidative stress markers. Moreover, in UC patients, they observed significantly enhanced salivary TGF-β1 levels, without any relationship with disease activity. It was explained that increased levels of TGF-β1 are associated with the defence stimulation of the differentiation of epithelial cells and the promotion of damaged mucosa repair.

Study of Said et al. [39] presented that oral dysbiosis was strongly associated with elevated inflammatory cytokines and lowered lysozyme in the saliva of IBD patients. The used Luminex technology allows measuring cytokines from little volumes of saliva samples with high sensitivity. Salivary concentrations of IL-6, IL-8 and MCP-1 were significantly higher in UC patients, while TNF-α was in CD patients. Additionally, in the saliva of both groups significantly elevated levels of IL-1β, IgA and LL37 (cathelicidin) were found. Cathelicidins are produced by the epithelial cells of mucosa in response to invasive bacterial infection [40]. In contrast, the salivary lysozyme level was significantly reduced compared to the healthy subjects. This antimicrobial protein (produced by macrophages, neutrophils and epithelial cells) plays a key role in the host constitutive defence system [41]. The lysozyme hydrolyses the cell wall of Gram-positive bacteria and may kill Gram-negative bacteria through synergic action with salivary lactoferrin [42]. Previous studies reported elevated levels of faecal lysozyme in IBD patients [61]. The alterations in salivary inflammatory biomarkers were strongly correlated with the abundance of four bacterial genera: *Streptococcus*, *Prevotella*, *Veillonella* and *Haemophilus*.

4. Conclusions

In conclusion, saliva contains several biomarkers (e.g., proteins or miRNAs) which can be used credibly to detect and control patients with Crohn's disease or ulcerative colitis. However, further investigations are necessary to validate these findings, as well as to identify new reliable salivary biomarkers for the early diagnosis and regular monitoring of inflammatory bowel diseases.

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