

Oral findings, Oxidative Stress and Antioxidant Biomarker Assessment in Serum and Saliva of Crohn's Patients

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Abstract: ***Background:** Crohn's disease (CD) is a transmural inflammatory disorder of any part of alimentary tract, mostly associated with the interaction of environmental risk factors and immunological disturbances to normal microbiota of the intestine. However in the recent year it has been proposed that oxidative stress as a result of immunological factors over activation was highly suggested to be one of the major pathological mechanisms of Crohn's disease. The natural cores of CD is fluctuate by periods of remission and flare up and associated with various intestinal and extra intestinal symptoms. **Subjects, Materials and Methods:** We aimed to compare the level of oxidative stress and antioxidants capacity in serum and saliva of fifty patients with moderate to sever Crohn's disease and twenty five control subjects by assessing lipid peroxidation Malondialdehyde (MDA) and antioxidant glutathione (GSH) markers. Blood and saliva samples were collected and salivary flow rate was measured for control subjects and Crohn's patients. Body mass index (BMI) was determined by dividing the weigh(kg) by length (m²) for all participants. **Results:** The mean of BMI of Crohn's patients showed non significant difference (p>0.05) than control subjects, while Crohn's patients showed highly significant reduction (p<0.001) in the means of salivary flow rate than that of control subjects. The results revealed that the levels of serum and saliva MDA were highly significantly higher (p<0.001) in Crohn's patients than that of control subjects, while highly significant reduction (p>0.001) in the levels of serum and saliva GSH was showed in Crohn's patients comparing with that in control subjects. The highest percentage of Crohn's patients presented with fungal infection as the most predominant oral findings among those patients. **Conclusions:** Chronic inflammatory characteristic of Crohn's disease was highly associated with a state of imbalance between oxidants and antioxidants system of the body, known as oxidative stress condition. Saliva acts as an important diagnostic felid as serum in detection of many systemic diseases. Patients with Crohn's disease were Immuno compromised as a result of disease associated immunological dysregulation or immunosuppressant medications usage and this increase the risk of opportunistic infection in Crohn's patients.*

Keywords: Crohn's disease, Oral findings, Oxidative stress (MDA), Antioxidant (GSH)

1. Introduction

Crohn's disease is a chronic relapsing inflammatory disorder of the gastrointestinal tract, that's mostly associated with immunological hyperactivity to commensal intestinal microflora which thought to be triggered by genetic predisposition and environmental risk factors[1]. Patients with Crohn's disease mostly presented with intestinal and extra intestinal symptoms. The most common intestinal symptoms of CD are abdominal pain, diarrhea mixed with blood or mucus or both, fever and weigh loss in a short period of time[2]. Skin, joints, eyes and oral cavity are frequently involved with the extra intestinal manifestations of Crohn's disease[3]. More than 80% of Crohn's cases presented with oral manifestations at early stage of the disease prior to intestinal involvement[3]. Cobble stoning of the oral mucosa, lip swelling, deep linear ulcerations, mucogingivitis, and tissue tags are considered as the most pathognomonic oral lesions of CD. Other nonspecific oral findings such as angular cheilitis, aphthous ulcerations, glossitis, stomatitis and perioral dermatitis can also seen in patients with Crohn's disease as a result of pathological process of the disease or as a consequence of nutritional deficiencies and medications side effects[4]. The decision of Crohn's disease management should be targeted the risk factors such as smoking cessation and encourage diet of high fiber and fruits[5]. A number of medications are prescribed to control the inflammatory process of CD according to severity of clinical course of the disease as

mild to moderate cases can be treated with corticosteroid and 5-aminosalicylates, while for sever cases it was preferred to intensify the treatment such as immunosuppressants (e.g. Azathioprine) and/or biological agents (e.g. anti TNF- α Infliximab)[6]. In Crohn's disease mononuclear cells (i.e. T lymphocyte) condensed in the intestinal mucosa due to impaired cellular apoptotic program secondary to disease associated changes such as increase concentration of TNF- α , a pro-inflammatory cytokine, which has an important role in cellular survival and apoptosis regulation. These immune cells and pro-inflammatory cytokines induce oxidative tissue damage in Crohn's patients by increase reactive oxygen species (ROS) production [7]. Free radicals and ROS are harmful substances with cytotoxic damaging effect of essential biomolecules of the body such as lipid, protein and DNA[8]. Harmful effect of oxidizing substrates could be neutralized by substances known as antioxidants [9]. It was suggested that inflammatory process of CD associated with increased oxygen species production and reduced antioxidants activity putting tissues under a state of oxidative stress[9].

2. Materials and Method

This study was performed in gastroenterology and hepatology teaching hospital in Baghdad city by enrolling fifty Crohn's patients with moderate to sever course of Crohn's disease without any other systemic diseases and twenty five healthy subjects. Body mass index (BMI) was

determined by weigh and length measuring of all participants then calculated by dividing the weigh(kg)by the square of length(m²), BMI=weight (kg) /height (m²). Oral cavity of each patient was examined carefully for detection the presence of primary or secondary associated oral manifestations by using dental mirror and probe, beginning with upper and lower lips, labial mucosa, right buccal mucosa and sulcus, right retro molar area, left buccal mucosa and sulcus, left retromolar area, dorsal surface, lateral margins and ventral surface of the tongue, floor of the mouth and lastly hard and soft plate. Then unstimulated saliva was collected, while the patients in a fasting state at least 90 minutes after the last meal, by spitting every 60 seconds in a collected tube. collection was continued for 10 minutes then salivary flow rate was calculated by dividing the collected volume (ml)by10 min. (time of collection), Saliva flow rate=volume of saliva (ml) \collection time (min).From antecubital vein 8 ml of blood was drawn by 10 ml syringe with 21 gaugesterile needle .Blood and saliva samples were centrifuged at 3000 rpm, then the top supernatants was withdrawn and distributed in 3 small tubes for each blood and saliva sample and kept at -20C° till the samples collection completed. Lipid peroxidation Malondialdehyde(MDA) in serum and saliva was measured spectro photometrically according to Buege and Aust[10].Serum and saliva antioxidant GSH was analyzed by depending the procedure reported bySedlack and Lindsay and modified by Al-Zamelyet al.[11].

3. Results

Age and gender:-This study included fifty Crohn's patient which consisted of 33 (66%) males and 17 (34%) females (M:F=1.9:1) with mean age±S.D. was (30.66±9.11)years and the age range (17-45)years. Control group consisted of age and sex matched 25 healthy subjects, 15(60%)subjects of them were males, while the remaining 10 (40%) were females with mean age±S.D. was(28.68 ±7.42) years, fig.(1).

Body mass index (BMI):-Statistical analysis of study parameters by using t-test revealed there was no significant difference (p>0.05) between the mean of BMI of Crohn's patients(24.46) and that of control subjects (25.46)(tab.1, 2).

Salivary flow rate:-The mean and S.D. value of saliva flow rate of Crohn's patients was (0.28±0.07) (ml/min), which was highly significantly decreased (p<0.001) than that of control subjects (0.40±0.08) (ml/min) (tab.1, 2).

Oral findings:-Findings of intraoral examination of Crohn's patients participated in this study revealed that candidiasis and angular chellitis(fungal infection), aphthus ulceration, dysphagia, hairy tongue, atrophic glossitis, lip swelling, lichen planus and desquamative gingivitis have been showed in the oral cavity of Crohn's patiens .The most predominantoral findings that noticed among all patients was fungal infection which presented in 24(48%) of them. Aphthus ulceration was the second most common oral finding that seen in 13 (26%), then followed by dysphagia in 6 (12%), hairy tongue in 5 (10%), lip swelling and atrophic glossitis in 2 (4%) and finally lichen planus and

desquamative gingivitis in 1(2%) of the examined patients (fig .2)

Oxidative stress Malondialdehyde (MDA) biomarker:-The results of this study showed that mean value of MDA in serum of Crohn's patients was (0.27µmol/L) and this highly significantly higher(p<0.001) than that of control subjects (0.16 µmol/L) .the same was shown between the mean of saliva MDA in Crohn's patients(0.22 µmol/L)and control subjects (0.12 µmol/L) (tab.1, 2).

Reduced Glutathione (GSH):-The mean of serum GSH in Crohn's patients (11.52 mmol/L) was highly significantly decreased(p<0.001) than that of control subjects (14.51 mmol/L), also highly significant reduction (p<0.01) was found between saliva GSH of Crohn's patients (10.32 mmol/L) and that of control subjects (12.88 mmol/L) (tab.1, 2)

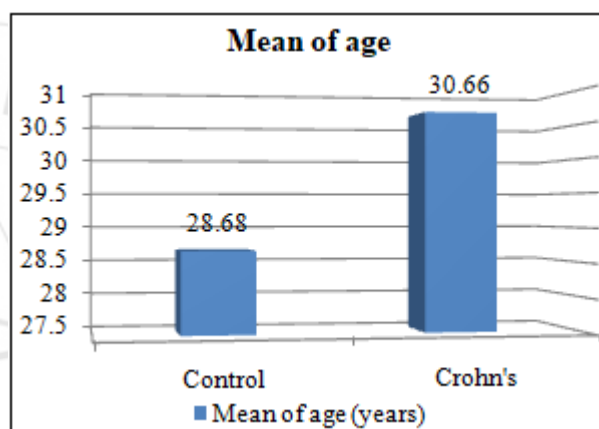


Figure 1: The mean values of age of healthy control and Crohn's patients

Table 1: The mean and S.D. values of all study parameters of both Crohn's and control groups

Parameters	Crohn's N=50		Control N=25	
	Mean	S.D.	Mean	S.D.
BMI (Kg/m ²)	24.46	±2.24	25.46	±2.59
Saliva flow rate (ml/min)	0.28	±0.07	0.40	±0.08
Serum MDA (µmol/L)	0.27	±0.06	0.16	±0.02
Saliva MDA (µmol/L)	0.22	±0.07	0.12	±0.03
Serum GSH (mmol/L)	11.52	±3.44	14.51	±2.76
Saliva GSH (mmol/L)	10.32	±3.33	12.88	±3.04
Serum V. E (µmol/L)	2.13	±8.75	3.17	0.95
Saliva V. E (µmol/L)	1.71	±0.55	2.40	0.67

Table 2: The t-test of all study parameters between Crohn's patients and control subjects.

Parameters	t-value	P-value	Sig.(2-tailed)
BMI(Kg/m ²)	1.72	0.08	NS
Saliva flow rate(ml/min)	6.26	0.000**	HS
Serum MDA (µmol/L)	8.31	0.000**	HS
Saliva MDA (µmol/L)	5.84	0.000**	HS
Serum GSH (mmol/L)	3.77	0.000**	HS
Saliva GSH (mmol/L)	3.21	0.002*	HS
Serum V.E (µmol/L)	4.44	0.000**	HS
Saliva V.E (µmol/L)	3.65	0.0004**	HS

NS:non-significant, HS:highly significant
 * P< 0.01, ** P< 0.001

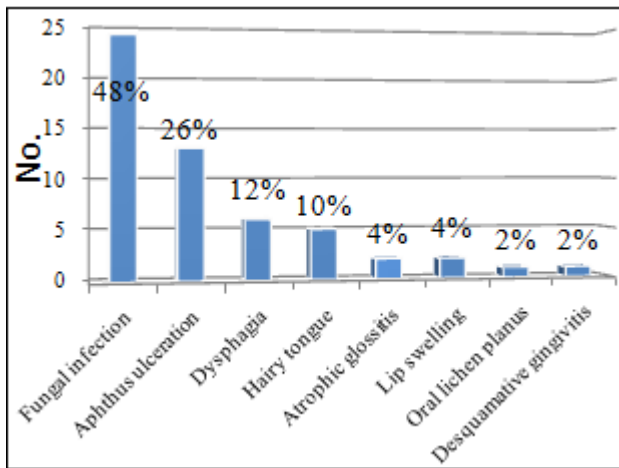


Figure 2: Oral manifestations of total Crohn's patients

4. Discussion

Age and gender: In this study the mean±S.D. age of participated Crohn's patients was 30.66±9.11 years with the range of age between 17 to 45 year. Crohn's disease in this study showed male predominance with male to female ratio (M:F=1.9:1), 33 (66%) were males and 17 (34%) were females .

Esmat *et al.*[12] found from 157 Egyptians patients with IBD, 22 subjects were diagnosed to have CD with the mean of ages was 29.7 years and male to female ratio was 2.6:1.

In 2014, the largest cohort epidemiological study of CD done by Aljebreen *et al.*[13] demonstrated increased prevalence of CD in Saudi Arabia and middle east countries with greater incidence in male (58.6%) more than female (41.4%) (M:F=10:7), in contrast to demographic data of studies from European and North American that showed females in these areas were more affected with CD .

Body mass index:-The results showed no significant difference was found between the mean of BMI of Crohn's patients and healthy control subjects, and this may be attributed to the effect of medical therapy in controlling inflammation and mucosal healing achievement which has been suggested to be highly implicated in nutritional status and intestinal absorption improvement, thus increase BMI.

Meta analysis that involved 1442 IBD patients and 2059 healthy control subjects done by Dong *et al.*[14] found that body mass index of Crohn's patients without medical therapy was significantly lowered than healthy control subjects due to reduced dietary intake and impaired intestinal absorption, while significant improvement of BMI has been detected in patients on medical therapy and showed non significant difference than control group due to relieve clinical symptoms of the disease that causing increase dietary intake and nutritional status enhancement .

Salivary flow rate:- The results of the present study showed that salivary flow rate of Crohn's patients was highly significantly lower ($p < 0.001$) than control subjects. Katz *et al.*[15] reported that the prevalence of dry mouth and decreased salivary secretion among patients with CD more than that in control subjects .This study showed that

granulomatous and immunological alterations affected salivary glands of Crohn's patients, were responsible of many complications such as total or partial parotid duct obstruction, ductal cyst formation and eventually coetaneous fistula formation and these complications were highly implicated in decreased salivary flow among Crohn's patients.

Oral findings:-Intraoral examination revealed that fungal infection was the main oral findings that seen among Crohn's patients of this study.

Dave *et al.*[16] and Rahier *et al.*[17] were mentioned that patients with IBD mostly treated by immune modulator agents including thiopurines, anti-TNF α biological agent, corticosteroid and methotrexate. These agents were reported to increase the risk of immunosuppressive effect and bone marrow suppression and mainly involved in rising opportunistic infections rate especially when used simultaneously. Aphthous ulceration also detected in those patients. This may be attributed to oral involvement with the inflammatory process of the disease or as a result of nutritional (iron, folic acid and vit. B12) deficiency and impaired immune system as a medication side effect [18]. Crohn's patients have been shown to suffer from dysphagia as chronic transmural inflammation of CD may be associated with fibrosis causing motility disorder or restricted mass of esophagus ;therefore, dysphagia and swallowing difficulty could be reported in Crohn's patients [19].

Oxidative stress Malondialdehyde (MDA) biomarker:-The mean of serum and saliva MDA of patients with Crohn's disease showed highly significant increasing ($p < 0.001$) than that of control subjects as chronic inflammation of intestinal mucosa in Crohn's patients associated with production excessive amount of reactive oxygen species (ROS) and increase oxidative stress (OXS) by infiltrated inflammatory cells (polymorphonuclear neutrophils and mononuclear cells) that are presented in the affected intestinal mucosa of Crohn's patients, as these cells in response to different stimulus are associated with releasing large amount of noxious substances including ROS and pro-inflammatory cytokines (IL-1, IL-8 and tumor necrosis factor alpha (TNF- α)) which in turn, enhance ROS production by inflammatory cells . Free radicals generated as a result of inflammatory process are mainly involved in massive tissue damage including cell membrane [20].

Antioxidants reduced Glutathione (GSH):-The results of the present study showed that serum and saliva GSH were highly significantly decreased in Crohn's patients than that in control subjects. Reduction of this enzymatic antioxidant may be related to imbalance between ROS - antioxidants levels, proposing that increased ROS and MDA generation as a result of tissue damage in CD, leading to excessive antioxidants consumption by the process of free radicals scavenging and this agreed with findings of Grisham *et al.*[21] who revealed that normal human colonic mucosa presented with fewer amount of enzymatic antioxidants compounds than that in other parts of the body and these amounts significantly decreased in cases of acute and

chronic inflammation of the intestine due to excessive exposure to oxidative stress.

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