

The Effect of Biological Drug on DNA Integrity in Psoriatic Patients

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Abstract: Psoriasis is an autoimmune and inflammatory skin condition with reactive abnormal epidermal differentiation disease in which genetic and environmental factors have a significant role. Therapeutic agents that either modulate the immune system or normalize the differentiation program of psoriatic keratinocytes are suggested for treating psoriasis. Based on the severity of the disease in patients, only patients with severe disease will be treated with biological therapy (Etanercept (ETN)) in this study. ETN is a new, safe and effective treatment for moderate to severe psoriasis. This type of medication is designed to target specific components of the immune system. Comet assay Single cell electrophoresis assay (SCGE) is a very sensitive method to determine DNA damage caused by exposure to mutagenic agents that effect in producing autoimmune disease. The aim of this project was to evaluate DNA damage in psoriatic patients treated with ETN. Blood samples were collected from forty-eight patients of males & females with severe psoriasis and fifty healthy people as control group; alkaline comet assay was applied for both two groups. The results showed that psoriasis patients (before and after treatment) had a significant higher DNA damage than in the control group. It was concluded that psoriatic patients had high score of comet assay and ETN has no effect on the DNA (damage or repair).

Keywords: Psoriasis, Etanercept, and Comet assay.

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I. Introduction

Psoriasis is a common, chronic, immune-mediated skin disease with systemic pro-inflammatory activation, where both environmental and genetic factors contribute to its pathogenesis (Trojacka et al., 2015). This disease usually occurs in the second-to-fourth decade of life, and both sexes are equally affected (Wang et al., 2015). The prevalence of psoriasis may vary from region to region due to variable environmental and genetic factors (Asokan et al., 2011). A systematic worldwide review found the prevalence of psoriasis ranged from 0.5 to 11.4 percent in adults and 0 to 1.4 percent in children (Michalek et al., 2017).

Psoriasis was considered to be a chronic inflammatory dermatosis with albeit, genetic factors involved in the pathogenesis. It is characterized by the presence of sharply demarcated, red plaques with adherent silvery-white scales and a tendency for symmetrical distribution over the body (Mehta et al., 2010). It is actually caused by a combination of both a primary defect in keratinocytes and an inappropriate innate and adaptive immune response– driven type I interferon (IFN- α) and that it is mediated mainly by resident and infiltrating T cells (Conrad et al., 2007).

The cause of the loss of control of keratinocyte turnover is unknown. However, environmental, genetic, and immunologic factors appear to play a role (Langley, 2012). There is a multifactorial pattern of inheritance. About 30% of patients with psoriasis have a family history (Rahman and Elder, 2005). Human body cells are constantly exposed to harmful factors which have the potency to cause DNA damage. Most of these harmful factors are of oxidative in nature (Kryston et al., 2011). The damage of DNA can be estimated by different ways, one of them is Comet assay Sunitha et al., 2008), or Single cell gel electrophoresis (SCGE), commonly known as the comet assay, DNA fragments of different lengths move differently in an agarose gel electrophoresis. The proportion of DNA in this fast-moving “comet tail” is an indication of DNA damage. DNA damage, as both single and double-strand breaks, is assessed at the level of individual cells (Tice et al., 2000, Dusinska and Collins, 2008).

Etanercept drug:

Etanercept (ETN) (Enbrel trade name) was the first TNF- α inhibitor to be approved for use in Psoriasis. ETN is a dimeric, soluble fusion protein consisting of the extracellular ligand binding portion of the TNF receptor linked to the Fc portion of human IgG1 (figure 1.6). It is capable of binding and neutralizing soluble TNF and transmembrane TNF (Kerensky et al., 2012). It is a soluble tumor necrosis factor receptor fusion protein that reversibly binds to tumor necrosis factor, (Giannini et al., 2009), furthermore, it alters neutrophil migration,

dendritic cell and T-cell maturation and migration, thus decreasing the local and systemic production of pro-inflammatory cytokines and their subsequent effects (Tracey et al., 2008).

II. Materials & Methods

Study design

This case-control study was carried out at the Laboratories of Biology department / University of Baghdad & Teaching Laboratories in City of Medicine.

Ethical consideration

The questionnaire has been designed to collect information directly related to the research questions, and no private or personal questions were asked from respondents. License were taken directly from the patients verbally.

Patients

A total of 48 Psoriatic patients that must be suffering from sever to moderate psoriasis disease were included in the present study. These patients attended to the Dermatology and Venereology Department in Baghdad Teaching Hospital during the period from December 2016 to June 2017. These patients stopped responding to all other treatments so they were diverted to take biological therapy ETN. Complete history was registered in an information sheet. It was prepared and designed according to a questionnaire including history of present illness.

Blood sampling

Five milliliters of blood were collected by venipuncture from all patients and control groups attending some private clinics. Each collected blood sample was dispensed into two tubes: heparinized tubes for complete blood picture and comet assay.

Comet assay

Comet assay was used to determine DNA damage. Comet assay kit was used to perform the test (Olive *et al.*, 1990; De Boeck *et al.*, 2000).

Comet assay (Single cell gel electrophoresis assay SCGE)

For analysis purpose, mainly the terms comet length (CL) and tail length(TL) were used. With the help of computerized image analysis systems, it is possible to analyze percentage of DNA in the tail (% of the tail DNA) and the tail moment (TM).Automated tools employ image analysis techniques to recognize and measure comets, and are generally much faster than manual scoring. Due to the efficiency gained through automation, one can typically afford to measure larger sample sizes, which is critical for statistically significant results(Sharma *et al.*, 2012) and these parameters have been widely used by many workers (Bajpayee *et al.*, 2002, Anderson *et al.*, 2003)for genotoxic studies.

The results showed that the percentage of tail DNA (TL) was significantly higher ($P<0.05$) in psoriatic patients before and after treatment($4.18 \pm 0.66 \mu\text{m}$ and $4.11 \pm 0.54 \mu\text{m}$) respectively incomparison to control group ($0.81 \pm 0.0097\mu\text{m}$), while, there was no significant differences ($P>0.05$) showed between psoriatic patients before and after treatment. Table (1).

However, in the current study TM in control group was approximately ($0.032 \pm 0.003 \mu\text{m}$) which was significantly ($P<0.05$) lower than psoriatic patients before and after treatment($1.78 \pm 0.17\mu\text{m}$ and $1.89 \pm 0.093\mu\text{m}$)respectively, the results revealed that there were no significant differences ($P>0.05$)between TM inpsoriatic patients before and after treatment. Table (1).

Table (1) Comet parameters in psoriatic patients and controls (Mean±SD)

Groups	Tail length μm (Mean±SD)	Tail moment μm (Mean±SD)
Psoriatic patients before treatment	4.18 ± 0.66 *	1.78 ± 0.17 *
Psoriatic patients after treatment	4.11 ± 0.54	1.89 ± 0.093
Healthy control	0.81 ± 0.0097	0.032 ± 0.003

In the present study, representative images of alkaline comet assay in control, psoriatic patients before and after treatment, are presented in figure (1).

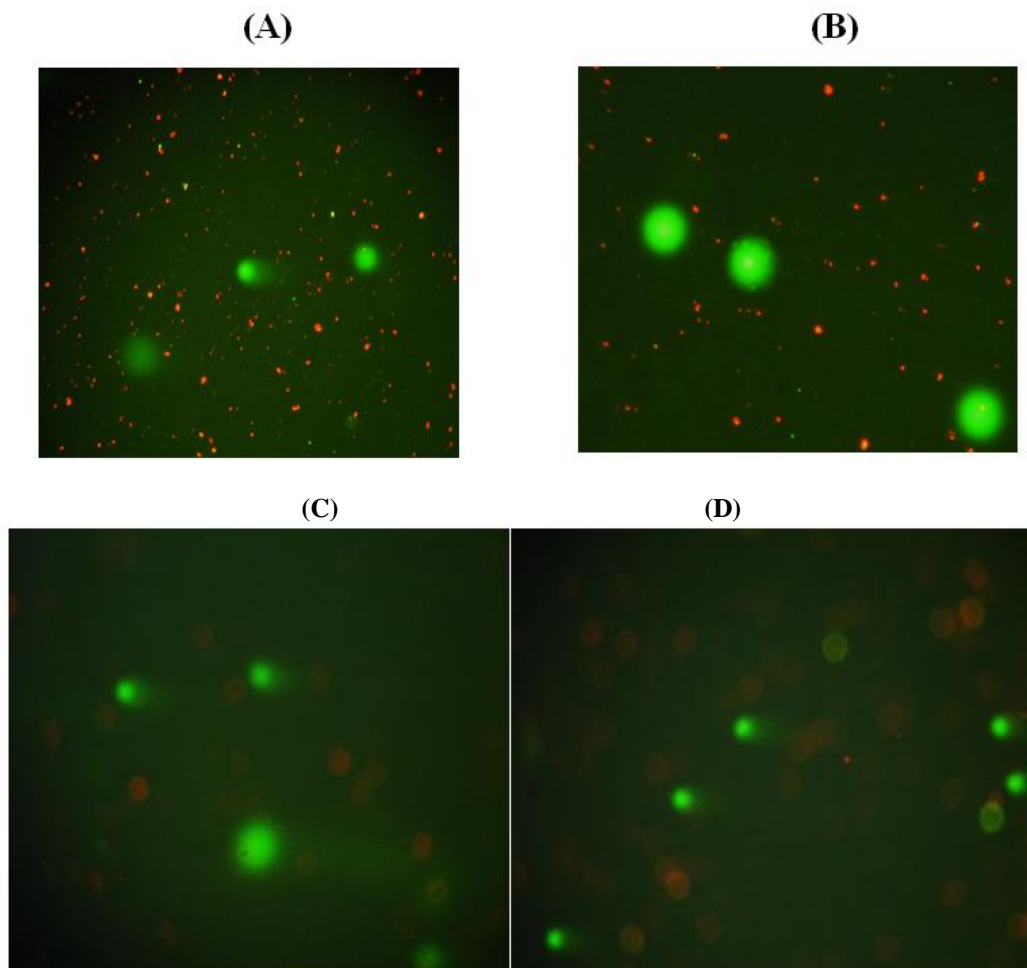


Figure (1): Assessment of DNA damage by alkaline comet assay in the blood lymphocytes in psoriatic patients: A: Compression between damaged and undamaged DNA, B: Undamaged DNA in healthy control group, C: Damaged DNA in psoriatic patients before treatment, D: Damaged DNA in psoriatic patients after treatment.

The current results were in line with (Møller et al., 1998), and Gandhi *et al.*, (2010) who indicated that more DNA damage determined by the comet assay in psoriatic patients in comparison with healthy control group. The explanation of this result may come from the fact that psoriasis is a chronic, inflammatory, proliferative skin disease it can be effected on any part of skin, especially on scalp and extensor prominences (Rachakonda et al., 2014, Kim et al., 2015). The skin is considered a potential target for oxidative injury, as it is usually exposed to reactive oxygen species (ROS) (Kim et al., 2014) (Lin and Huang, 2016). The comet assay is a method that measures oxidative stress (Chan et al., 2013, Sanchez-Quesada and Perez, 2013).

Previous study showed that plasma membrane of skin cells in the psoriatic lesions have a significant increase in the arachidonic acid which is the natural substrate for synthesis Malondialdehyde (MDA), an end product of lipid peroxidation (Ayala et al., 2014), and this oxidative stress playing a critical role in the pathogenesis of psoriasis (Peluso et al., 2016, Liu et al., 2017). ROS mediated oxidative damage involves a number of biological molecules since it causes DNA modification (Gupta et al., 2014), DNA strand breakage, oxidation to 8-oxo-2 deoxyguanosine, and DNA adduct (Fu et al., 2012), lipid peroxidation (Mihalas et al., 2017), and secretion of inflammatory cytokines (Chahbouni et al., 2017).

The increased of DNA damage that assessed by using comet assay revealed the increase the severity of psoriasis disease in the patients comparing with healthy control group, that means the severe or moderate cases of psoriasis will expose to more oxidative stress than healthy cases due to skin lesions (Cabarkapa et al., 2014, Gandhi et al., 2015).

DNA damage was assessed by alkaline comet assay which was relatively simple, versatile and inexpensive. The researchers can use this method in all areas of medicine in which oxidative stress plays a role (Cabarkapa et al., 2014, Gandhi et al., 2015). The comet assay is based on the ability of negatively charged fragments of DNA to be drawn through an agarose gel in response to an electric field. The extent of DNA migration depends directly on the DNA damage present in the cells (Azqueta et al., 2011).

The use of alkaline conditions for DNA unwinding and electrophoresis ($\text{pH} \geq 13$) were incorporated later (Singh et al., 1988), allowing the detection of double, and single-strand breaks, in addition to expression of alkali labile sites. widespread acceptance of this technique and extensive application of the same in various disciplines subsequently led to the establishment of guidelines for its use (Tice et al., 2000). The alkaline comet assay can be applied to measure DNA damage at the single cell level since it requires only a relatively small cell population. In addition, it is a simple, robust technique, capable of detecting low levels of DNA damage (Collins, 2004). It can detect DNA damage equivalent to as few as 50 single-strand breaks per cell and varying number of double strand breaks (Singh et al., 1988). The assay is able to measure both single- and double-strand breaks (Calini et al., 2002).

Compared to the various techniques, studies have shown that the Single cell Gel Electrophoresis or the Comet assay is highly sensitive method to detect low levels of DNA damage. Further the results can be obtained in a relatively short period of time even with less number of proliferating/non-proliferating cells. In addition, deployment of wide range of cells; peripheral blood mononuclear cells, buccal epithelial cells, nasal epithelial cells, lens epithelium, tear duct epithelial cells, sperms as well as biopsy tissues in comet assay makes it a versatile and potential tool of choice to assess the DNA damage and repair efficiency (Liao et al., 2009).

There are about seventeen different types of parameters to explain the comet features; among them the tail parameters metrics are the most frequently used, and they were expressed in micrometer. The tail parameters are the tail length, the tail DNA and the tail moment (Olive et al., 1992). Tail Length is the distance of DNA migration from the body of nuclear core and it is used to evaluate the extent of DNA damage. Tail moment (TM): It is defined as product of tail length and the percentage DNA in tail. TM incorporates a measure of both the smallest detectable size migrating DNA (reflected in the comet tail length), and the number of relaxed / broken pieces (represented by the intensity of DNA in the tail) (Collins, 2004).

The current results disclosed that, there was no damage or repair in DNA molecule was shown by using ETN drug as monotherapy, figure (1). Treatment of psoriasis depends on the extent manifestations. Light-mild symptoms may respond to physiotherapy and non-steroidal anti-inflammatory drugs, while more severe diseases are requiring treatment with corticosteroids or disease modifying drugs (Mease, 2002). Much attention has been focused on agents that inhibit the activity of pro-inflammatory cytokines, which are believed to play a certain role in the disease outcome. ETN is one of these agents which is considered a TNF- α antagonist used in treatment of auto-immune disease (Kerensky et al., 2012). Given its dimeric structure, each ETN molecule can bind up to two TNF- α molecules receptors, compared with the monomeric endogenous TNF- α receptor, which can only bind one TNF- α molecule (Nesbitt et al., 2007), but it formed relatively unstable complexes (Scallon et al., 2002).

Linkage of the receptor to the Fc portion of IgG1 also substantially prolongs the half-life of ETN relative to the endogenous soluble forms (Nesbitt et al., 2007). It reduces the effect of naturally present TNF- α , and hence is a TNF- α inhibitor, functioning as a decoy receptor that binds to TNF- α (Zalevsky et al., 2007).

TNF- α is a cytokine produced by lymphocytes and macrophages. It mediates the immune response by attracting additional white blood cells to sites of inflammation, and through additional molecular mechanisms which initiate and amplify inflammation. There are two types of TNF- α receptors: those found embedded in white blood cells that respond to TNF- α by releasing other cytokines, and soluble TNF- α receptors which are used to deactivate TNF- α and blunt the immune response. In addition, TNF- α receptors are found on the surface of virtually all nucleated cells (red blood cells, which are not nucleated, do not contain TNF- α receptors on their surface). ETN mimics the inhibitory effects of naturally occurring soluble TNF- α receptors, the difference being that ETN, because it is a fusion protein rather than a simple TNF- α receptor, has a greatly extended half-life in the bloodstream, and therefore a more profound and long-lasting biologic effect than a naturally occurring soluble TNF- α receptor (Madhusudan et al., 2005).

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