ORIGINAL ARTICLE

Descriptive Cross-sectional Study of Bcl2 & p53 Expression in patients with Generalized Vitiligo at Arar city of Northern Saudi Arabia

DHAIFALLAH A. ALENEZI
Department of Dermatology Faculty of Medicine, Northern Borders University in Arar. Kingdom of Saudi Arabia
Correspondence author: Dr. Dhaifallah A. Alenezi Email: Drdhaif@hotmail.com, 00966556915555

ABSTRACT

Background: The mechanism behind vitiligo has been vague with most of studies reported different findings and conclusions. In Northern borders region of Saudi Arabia, vitiligo became highly prevalent and has great effect socially among population.
Aim: To investigate the expression of Bcl-2 and p53 in order to understand the mechanism behind vitiligo.
Method: descriptive cross-sectional study conducted at a specialized dermatology center in the city of Arar, Northern Borders of Saudi Arabia. Biopsies were taken from 50 patients (30 males, 20 females) along with 20 healthy control and investigated for the expression of Bcl-2 and p53 using immune-histochemical technique.
Result Bcl-2 expression as anti-apoptotic markers was high in skin of normal subjects with while there was no expression within the lesion of all fifty subjects, p53 has shown very low expression (+) in all 20 normal skin control subjects while of the 50 vitiligo subjects the expression varied between (++) within peri-lesional area and to within the lesional area with no significant gender or age variation.
Conclusion: our findings regarding Bcl-2 and p53 expression correlates with previous findings reported within the literature and strongly support the hypothesis of apoptosis as a major mechanism behind vitiligo more than necrosis.
Keywords: Vitiligo, apoptosis, Necrosis, Bcl-2, P53

INTRODUCTION

Vitiligo is a chronic pigmentary condition characterized by depigmentation patches on skin due to death or dysfunction of melanocyte cells responsible of giving the skin its uniform color[1]. Clinically, vitiligo appears as white macules on the skin could be locally or generalized all over the body with its spreading patterns are un-determined. Normally, melanocytes are the cells within the skin responsible of producing melanin which gives the skin its uniform color, they originate neural crest cells (NCC) and spread in different parts of the human body include inner ear, nervous system and heart [2,3]. The life cycle of melanocytes start from specification from embryonic crest cells in form of (melanoblast), migration and proliferation of melanoblast, differentiation of melanoblast into melanocytes which in turn mature into melanin producing organelles called melanosomes where it will be carried to reach the keratinocytes[4]. Keratinocytes form (85-90%) of the keratinizing epithelium of the edpidermis while non-keratinocyte cells form only (10-15%) of the cell population, the ration of melanocytes to keratinocytes depends on their location with the lowest being approximately 1:4 in cheek to the highest of 1:11 in the thigh[5]. In routine tissue section, melanocyte are commonly identified by antibodies to MART-1 protein[6,7,8].

Bcl-2 oncoproteins are expressed in Melanocytes to prevent cells from undergoing pre-mature apoptosis[9]. Causes lead to vitiligo apart from exposure to certain chemicals is yet viruses and oxidative stress with any of them yet to be determined as the leading cause[10]. Mechanism by which melanocyte cell loss takes place are determined to be either apoptosis or necrosis[11], with apoptosis is thought to be more responsible than necrosis. P53 is a cellular gatekeeper gene acts as a guard to the cell from unexpected damage, it functions as tumor suppressor gene and regulate cell division and proliferation through different mechanisms including cellular arrest and signaling apoptosis at G2 point[12]. Apoptosis defined as the programmed cell death[13]. The wisdom behind apoptosis is to remove cells that encountered serious faults which can lead to formation of tumors, therefore, the process is done with highly precise selection order with no immune response unlike necrosis which occurs in form of a traumatic cell death result in immune response[14]. Apoptosis could be initiated through one of two pathways, the first one is known as intrinsic pathway where the cell kill itself due to stress, the second one the cell undergoes apoptosis due to external signal from other cells with both pathways activate caspases which are protease enzymes that degrades protein[15]. Epidermal apoptosis is subject to certain factors such as ultraviolet light B (UVB) radiation which causes sunburn cells[16], those factors may result in Bcl-2 suppressed expression or increase expression of p53 which in turn shield such people against cancer induced by UV light but similarly make them at risk of vitiligo.
In this study we aim to look at the expression of apoptotic genes in patients with vitiligo from the Northern borders region of Saudi Arabia. The area itself is a desert region with temperature in summer exceeds 50 ° C, which make people prone to sunburns.

PATIENTS AND METHOD

This is a descriptive cross-sectional study conducted at Alkhebra dermatology center in Arar Northern borders of Saudi Arabia. Fifty biopsies were collected by a pathologist for 30 males and 20 females aged between 18-43 years old along with 20 biopsies taken from normal subjects as control were prepared for histopathology analysis. They

were fixed by formalin (10%) and processed then stained for hematoxylin and eosin stain. Immuno-histochemical detection of Bcl-2 and p53 was performed using commercially available monoclonal antibody from ABCAM, detections of antibodies was demonstrated by using labeled streptavidin biotin ABCAM kit, which consists of secondary biotinylated goat anti-mouse antibody and conjugated streptavidin horse reddish peroxidase followed by 3',3'-Diaminobenzidine (DAB) chromogen.

Sections of (3-4 µm) thickness were embedded on frosted end slides, and were deparaffinized in xylene twice for 2 minutes. Rehydration was done using serial dilution of alcohol (100%, 90%, 80%, and 70%) respectively and placed in water (2 minutes each step), followed by treatment under pressure in the reveal solution for 2 minutes in the Deckloking chamber (ABCAM Company) in order to retrieve the antigens, block endogenous biotin, and clearing. Sections left to cooled in room temperature then incubated with 3% hydrogen peroxide in methanol in order to block the endogenous peroxidase activity for 5 minutes and then washed in Phosphate Buffered Saline (PBS).

According to the manufacturer's instructions, sections were incubated (1 hour at room temperature) with anti- Bcl-2 and Anti- p53 which was diluted by 1:10 using antibody diluent. Sections were washed in PBS and incubated with biotinylated secondary antibody for 15 minutes at room temperature, then washed well with PBS, and then incubated with streptavidin horse reddish peroxidase for 15 minutes at room temperature and washed well with PBS. 3,3'-Diaminobenzidine chromogen was applied for 2 minutes until the desired color intensity was developed according to the manufacturer instructions, and then washed with tap water to stop the reaction. Throughout the study, sections of tonsils and appendicitis tissues known to express the investigated proteins were analyzed in parallel to serve as positive controls. Omission of the primary antibody from these samples was implicated as a negative control. All sections were counterstained with hematoxylin and examined under light microscopy.

RESULT

To interpret results of slide for each patient, 5 samples of healthy patients were collected and assessed for Bcl-2 and p53 expression. With a maximum of 100 and at least 50 of Bcl-2 and p53 stained cells were scored as positive with only definite and unambiguous stain was accepted. Mean and standard deviation for positive control were calculated and patient's samples were considered positive if were > (mean + 2SD) of positive control sample.

All 70 sections were blindly scored by pathologist, the level of expression is represented by crosses range from one cross (+) which indicates weak expression and four crosses (++++) to represent the strong expression, while negative expression was represented by (-). Bcl-2 expression as anti-apoptotic markers was high in skin of normal subjects with no significant gender or age variation, while there was no expression within the lesion of all fifty subjects with vitiligo with no significant gender or age variation, in contrast to Bcl-2, p53 has shown very low expression (+) in all 20 normal skin control subjects while of the 50 vitiligo subjects the expression varied between (++) within peri-lesional area and +++ to ++++ within the lesional area with no significant sex or age variation.

Fig. 1. Expression of Bcl-2 within the normal skin

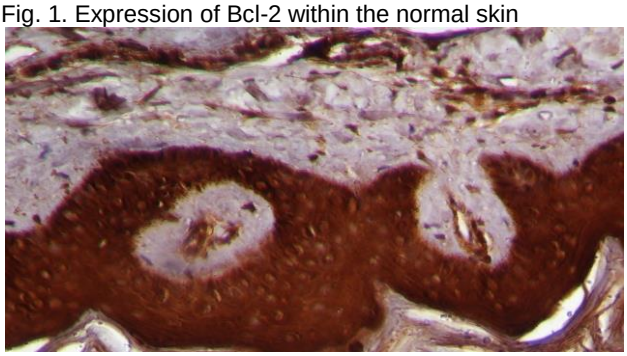


Fig. 2: Expression of Bcl-2 within the vitiligo region

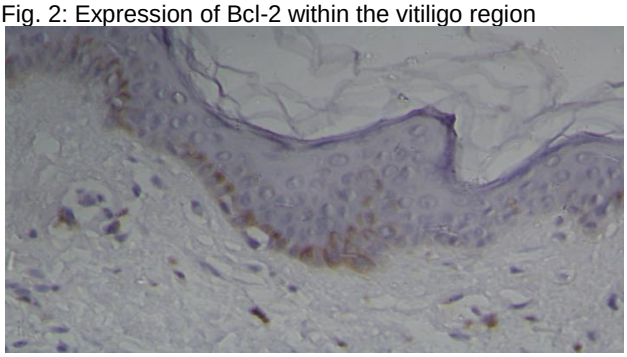


Fig. 3: Expression of p53 in normal skin

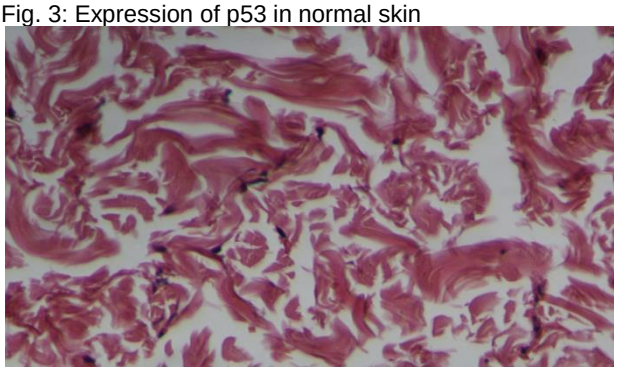
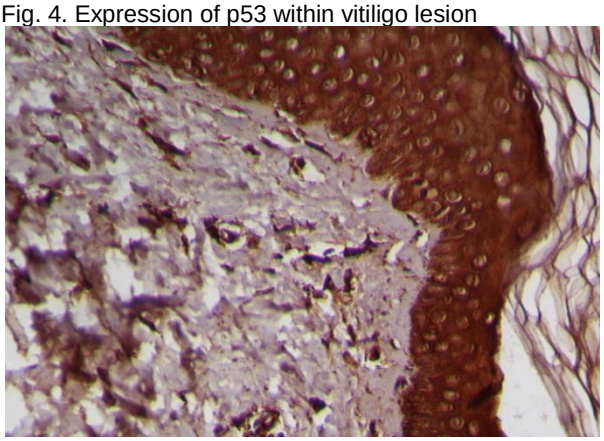


Fig. 4. Expression of p53 within vitiligo lesion



DISCUSSION

Bcl-2 (B-cell lymphoma 2) gene is a regulator gene and a portion of the Bcl-2 which responsible of regulating and promoting cellular apoptosis therefore it has been classified as an oncogene[17]. In autoimmune diseases, apoptosis has a significant role by producing unresponsiveness to selfantigen by either peripheral or central tolerance, while it can contribute to the etiology of some autoimmune diseases when it lack of activity[18].

In this descriptive study we aimed at analyzing the expression of Bcl-2 and p53 in vitiligo patients to shed the light over the mechanism of disease in Arar city of Northern Saudi Arabia, 100% of our patients have shown negative expression for Bcl-2 within their lesional area. Plettenberg and co, 1995 stated that melanocytes are the only cell type within human skin epidermis to express Bcl-2 at higher levels in both benign and malignant conditions, while in our findings the expression seems to have similar findings as the one reported by Ahmed M. Abdel-Aal et.al in 2002 among Egyptian population[19]. Bcl-2 was investigated because of its ability to regulate apoptosis while p53 was mainly investigated due to its role in regulating Bcl-2 expression and inducing apoptosis, the high expression of p53 within the lesional area compared to those of normal skin combined by down-regulation of Bcl-2 within the same lesional area very much support susceptibility of melanocytes to undergo apoptosis because of the loss of Bcl-2 preventive mechanism, our findings are also similar to Campbell et al, findings who reported upregulated p53 expression in ultraviolet light exposed human skin[20]

CONCLUSION

This study strongly conclude that the loss of the preventive property of Bcl-2 combined with the apoptotic inducing power of p53 is one of the major mechanisms behind vitiligo, furthermore treating patients with vitiligo using drugs that enhance the expression of antiapoptotic proteins such as Bcl-2 and inhibit p53 activity to prevent apoptosis may be of a great benefit to stop progression of the condition and pre-mature melanocytes death.

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