MAST CELLS AND APOPTOTIC BODIES IN SEBORRHEIC KERATOSIS: A COMPARATIVE STUDY BETWEEN UVB INDUCED SEBORRHEIC KERATOSIS IN MICE AND SPORADIC CASES IN HUMANS

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ABSTRACT

Background

Exposure to ultraviolet type B (UVB) radiation induces a number of pathologic changes in skin, including erythema, edema, epidermal hyperplasia, sunburn cell formation, immune suppression and eventually leads to cancer development.

Objective

To elucidate the differences in histological appearances of mast cells and apoptotic bodies between the two species (mice and human) among hyperkeratotic and acanthotic types of seborrheic keratosis (SK).

Materials and methods

Thirty paraffin blocks were used in this study; fifteen histologically confirmed acanthotic and hyperkeratotic SK cases in human (9 acanthotic and 6 hyperkeratotic) and fifteen blocks from both types acanthotic and hyperkeratotic SK cases in mice induced by UVB light (9 acanthotic and 6 hyperkeratotic).

Results

Our results revealed that there was a significant correlation between mast cells and apoptotic bodies for both groups according to Pearson Correlation test. In human cases mast cells counting ranged between 2-10 with a mean number of 5.2/1HPF, while the total number of apoptotic bodies ranged from 1-4 with a mean number of 2.6/10HPF. When compared to mice cases, the number of mast cells were increased with a range of 12-23 and with a mean number of 19.067/1HPF, while apoptotic bodies were decreased with a range of 3-20/10HPF and with a mean number of 9.4/10HPF.

Conclusion

Dermal mast cells infiltration were remarkably increased in mice skin specimens which were exposed to UVB. The number of apoptotic bodies in UVB induced cases were more than in human sporadic cases.

***Keyword*:** *UVB, seborrheic keratosis, apoptotic bodies, mast cells.*

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INTRODUCTION

The skin is the primary barrier against the external environment. The epidermal cells are the first cells to be exposed to physical and chemical genotoxic agents such as ultraviolet (UV) and ionizing radiations (IR) [1]. UVB radiation induces a number of pathologic changes in skin, including erythema, edema, epidermal hyperplasia, sunburn cell formation, immune suppression and changes in expression of numerous genes associated with proliferation, differentiation and skin cancer [2-3].

SK are the commonest benign epithelial tumors in older people; their prevalence increases with age [4]. SK are detected in 80-100% of people over 50 years and are typically localized on the head, the trunk and the extremities with the exception of the palms and soles. SK develop from the proliferation of epidermal cells [5-6]. The etiology of SK is still controversial, numerous studies regarded as of unknown etiology [7,8], not related to sun exposure [9-14], viral etiology [7, 15], or due to a mutation of a gene coding for a fibroblast growth factor receptor (FGFR3) [5,16]. However two previous studies induced SK in mice by UVB exposure [2,17].

One of the hallmark events of exposing skin to UVB radiation is the formation of sunburn cells (SC) [18], which are crucial protective mechanisms against the carcinogenic effects of UVB irradiation [19]. The formation of sunburn cells in UV-exposed skin indicates the severity of DNA damage. The repair of DNA damage in UVB exposed skin cells can prevent further damage. If they are not repaired, they may continue to replicate and may lead to cutaneous malignancies [20].

UV is also immunosuppressive and numerous studies have shown that UV-induced immune suppression is a major risk factor for skin cancer induction [21]. The exact process by which mast cells contribute to immune suppression is not known. Mast cells have been found to play a critical role in the suppression of immune reactions and not only through production of inhibitory cytokine interleukin 10 (IL-10). Thus, mast cell infiltration into tumor may possibly remodel tumor microenvironment and profoundly influence tumor behavior by participating and regulating inflammatory and immune reactions [22]. The present study was designed to elucidate the differences in histological appearance of mast cells and apoptotic bodies between the two species (mouse and human) among different types of SK.

MATERIALS AND METHODS

Study area

This study was conducted at the Pathology Research laboratory/Department of Pathology and Forensic Pathology/School of Medicine-University of Sulaimani where block sectioning, H&E staining and Giemsa staining were performed.

Sample collection:

A retrospective study was performed utilizing the paraffin embedded tissue blocks of both human and mice cases.

Human cases

Fifteen histologically confirmed acanthotic and hyperkeratotic SK cases were retrieved from the histopathology files of both Sulaimani Teaching and Shorsh Hospitals (9 acanthotic and 6 hyperkeratotic). Informed consent had been obtained from all patients according to the guidelines of the local ethics.

UVB irradiation group

The mice were subjected to UVB irradiation with a calculated power of 53 mj/sec using a lamp of 312 nm wavelength, 15 watts; Vilber-Lourmat-France. The mice were exposed to UVB light for 4days/week for 20 minutes repeated for 5 months. This was done after making a window by shaving the mice's back (5X2 cm).

Mice cases

Fifteen SK cases of specimens that induced by UVB exposure were collected from the archives of one previous study on mice skin.[2] All cases (Human and Mouse) were reviewed to confirm the diagnosis and assessed for suitability for our project. Two sections of 5µm thickness were taken from each paraffin embedded tissue block. The first section was mounted on a slide for Haematoxylin and Eosin staining for apoptotic bodies counting. The second section was for Giemsa stain (ARTISAN, DAKO COMPANY, DENMARK), for mast cells counting following the protocol that was supplied with the kit.

Cell counting

In this study the total number of mast cells was calculated as a mean number in 1 high power

field according to the mean calculation, while for apoptotic bodies; the numbers of apoptotic body in 10 high power fields were counted.

Statistical analysis

The data obtained from our observations were analyzed using ANOVA, Duncan's test and Pearson's Correlation.

RESULTS

Mouse Samples

The results revealed that there was a strong effect of chronic UVB irradiation in mice group by increasing the number of mast cells with a range of 12-23 and with a mean number of 19.067/1HPF (Figure 1,2). while, apoptotic bodies were decreased with a range of 3-20/10HPF with a mean number of 9.4/10HPF (Figure 3,4), a P-value of 0.0001 (according to F test) and with A-B symbols (according to Duncan's test). This indicates a highly significant difference between each parameter.

Human samples

The result showed there were no big differences among human cases for both parameters (mast cells and apoptotic bodies). Mast cells counting ranged between 2-10 with a mean number of 5.2/1HPF (Figure 5,6), while the total number of apoptotic bodies ranged from 1-4 with a mean number of 2.6/10HPF (Figure 7,8). There was no significant relationship between mean number of mast cells and total number of apoptotic bodies (according to F test) and with A-A symbols (according to Duncan's test).

Difference in correlation between total number of apoptotic bodies/10HPF and mean number of mast cells/1HPF in human and mouse cases

Results showed a strong inverse correlation between apoptotic bodies and mast cells in both groups i.e. increasing the number of mast cells with a mean number of 19.066 related to decreasing numbers of apoptotic bodies with a mean of 9.4 in mouse cases. While, for human cases slightly increasing number of mast cells with a mean numbers of 5.2 related to decreasing numbers of apoptotic bodies with a mean of 2.6 (Figure 9), and a P-value of 0.01 (F test) which indicate a highly significant inverse correlation between the number of mast cells and the number of apoptotic bodies. This is due to the effect of UVB irradiation in mouse cases and in human cases.

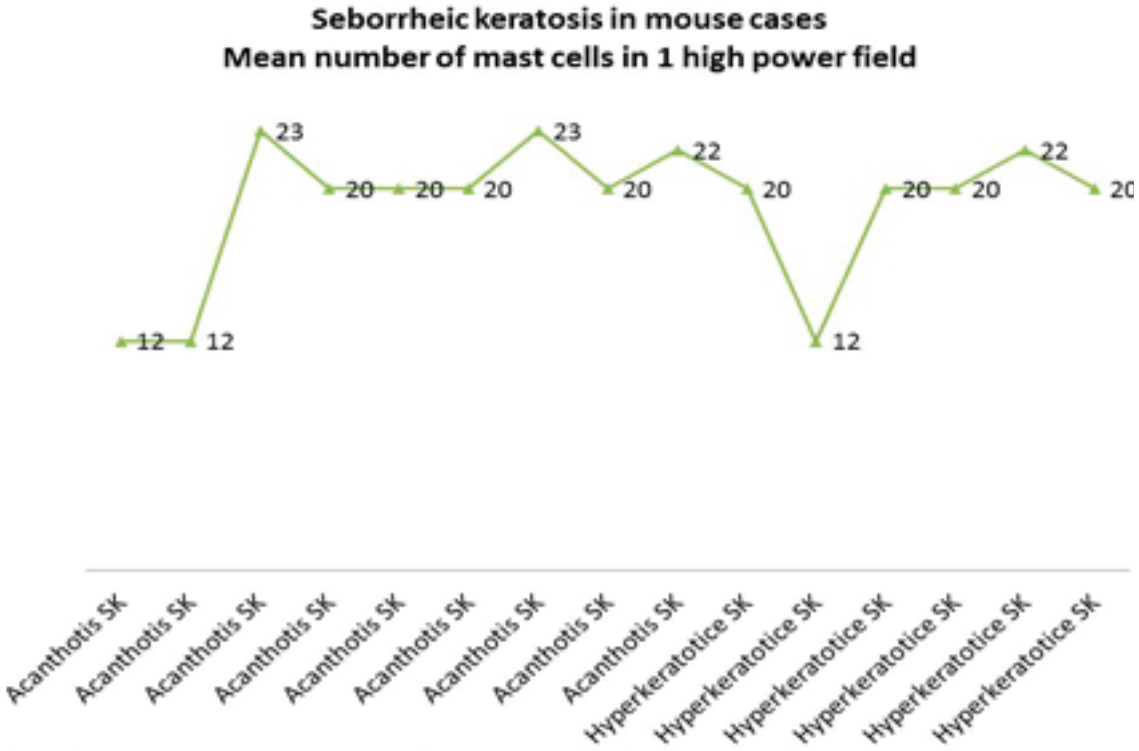

Figure 1. Line chart showing the mean number of mast cells in mouse group.

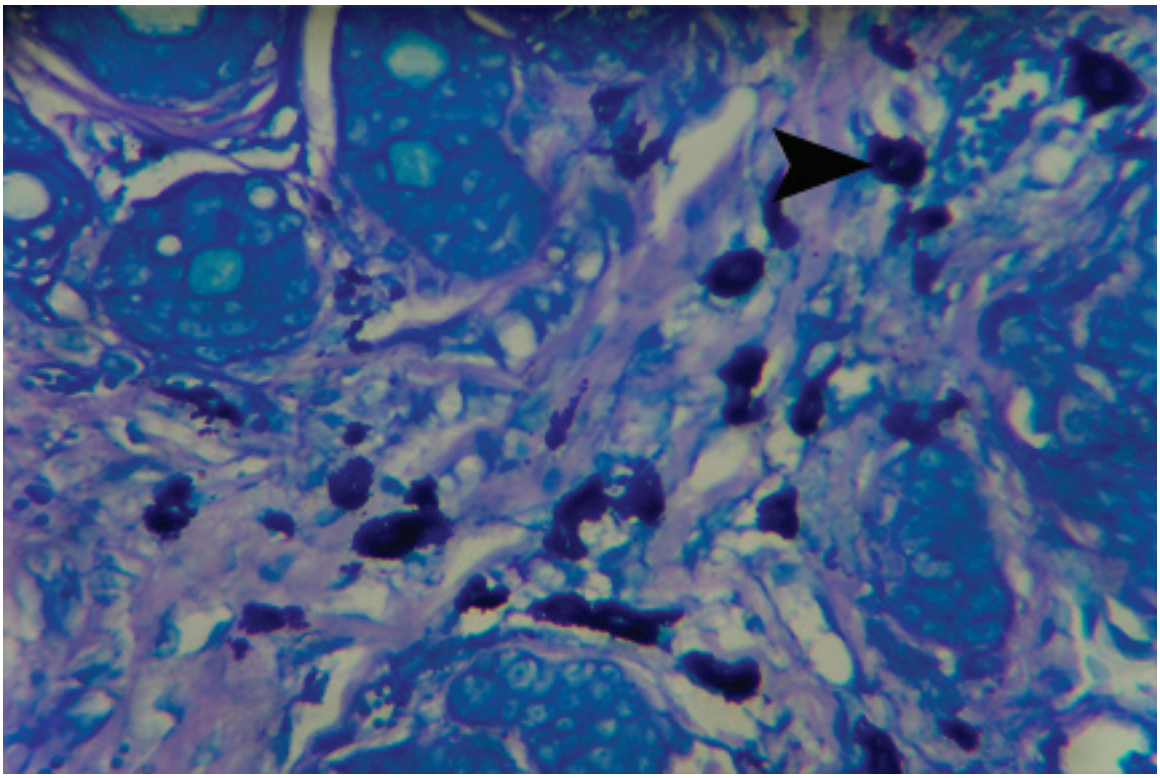

Figure 2. Microscopic features of pleomorphic hyperchromatic (violet) mast cells (Arrow head, Giemsa stain X400).

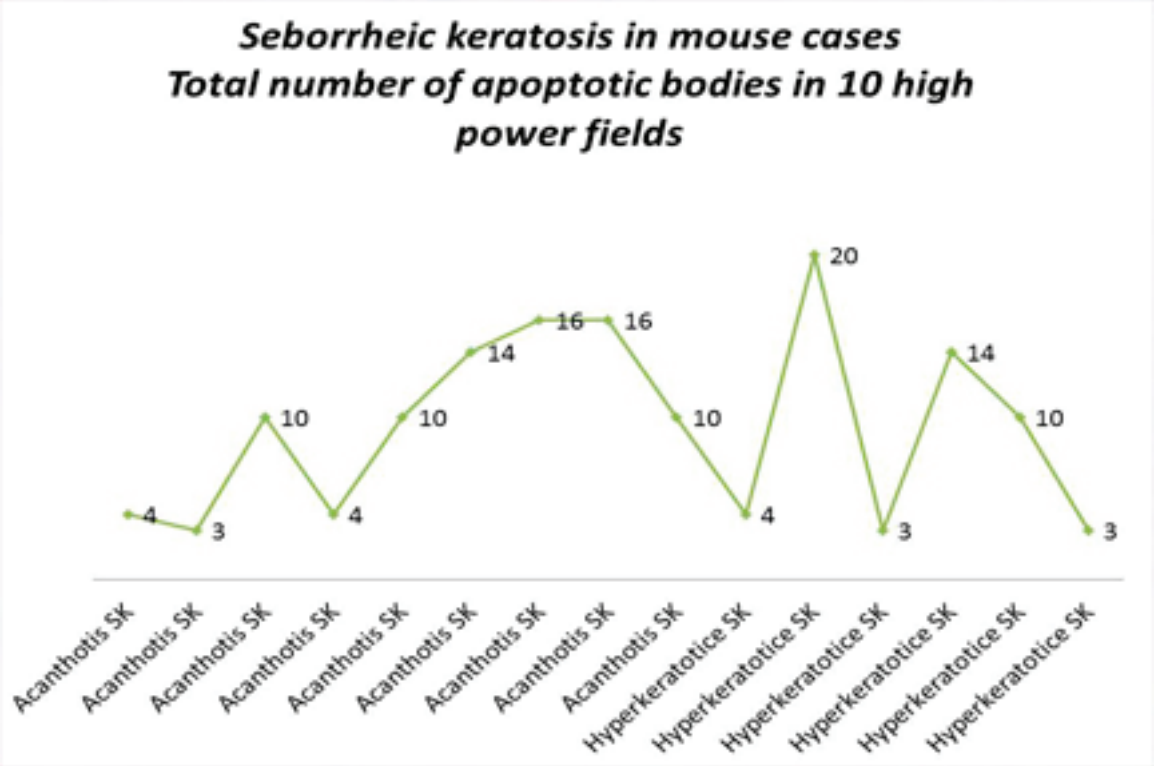

Figure 3. Line chart showing the total number of apoptotic bodies in mice group.

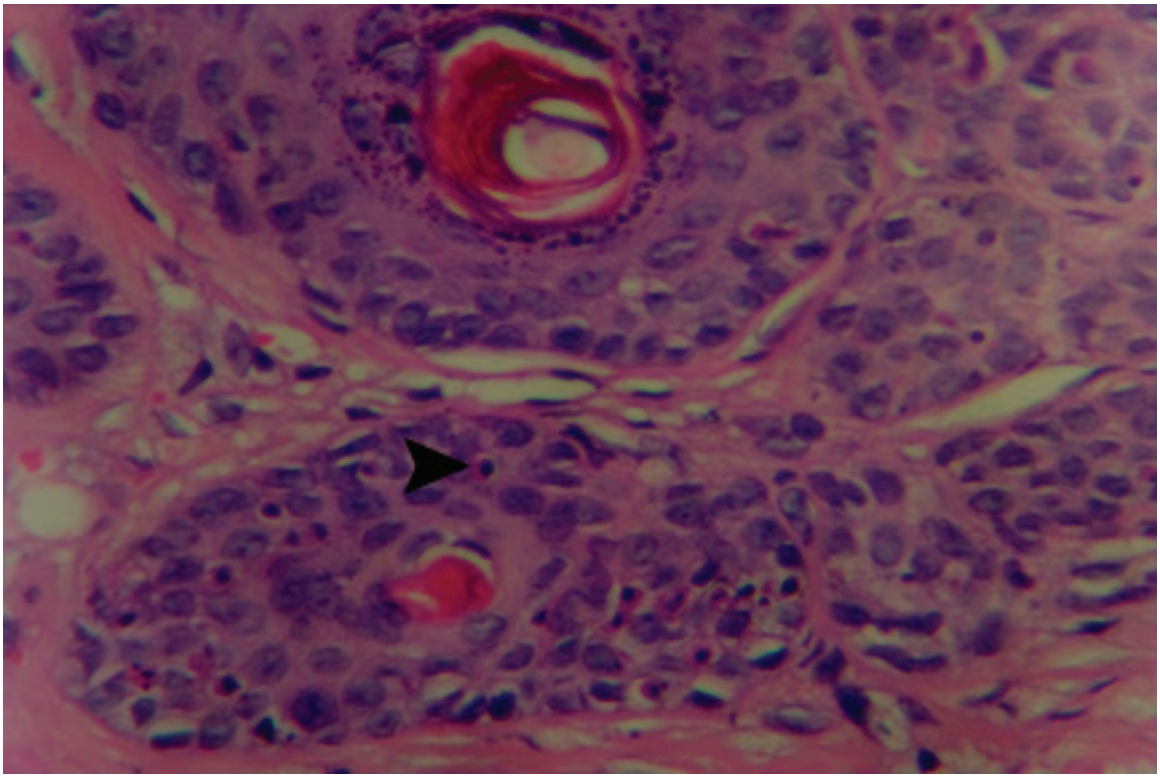

Figure 4. Microscopic appearance of apoptotic bodies (Arrow head, H&E stain X400).

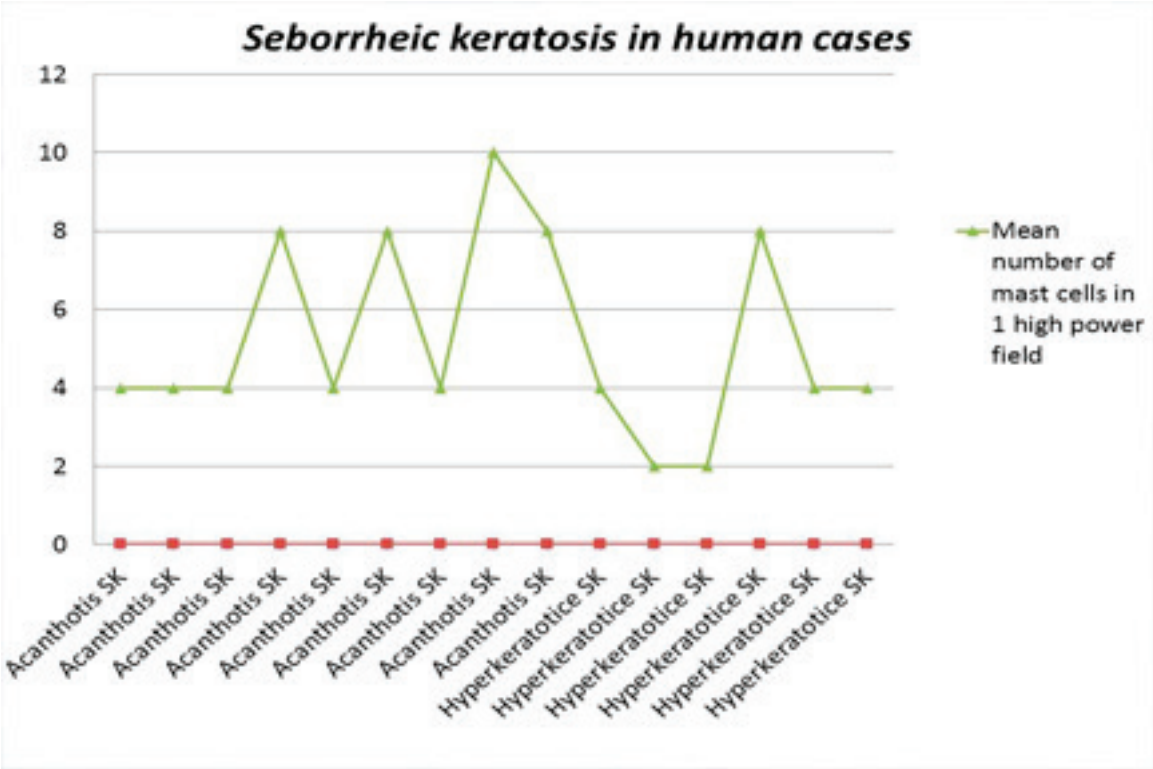

Figure 5. Line chart showing the mean number of mast cells in human group.

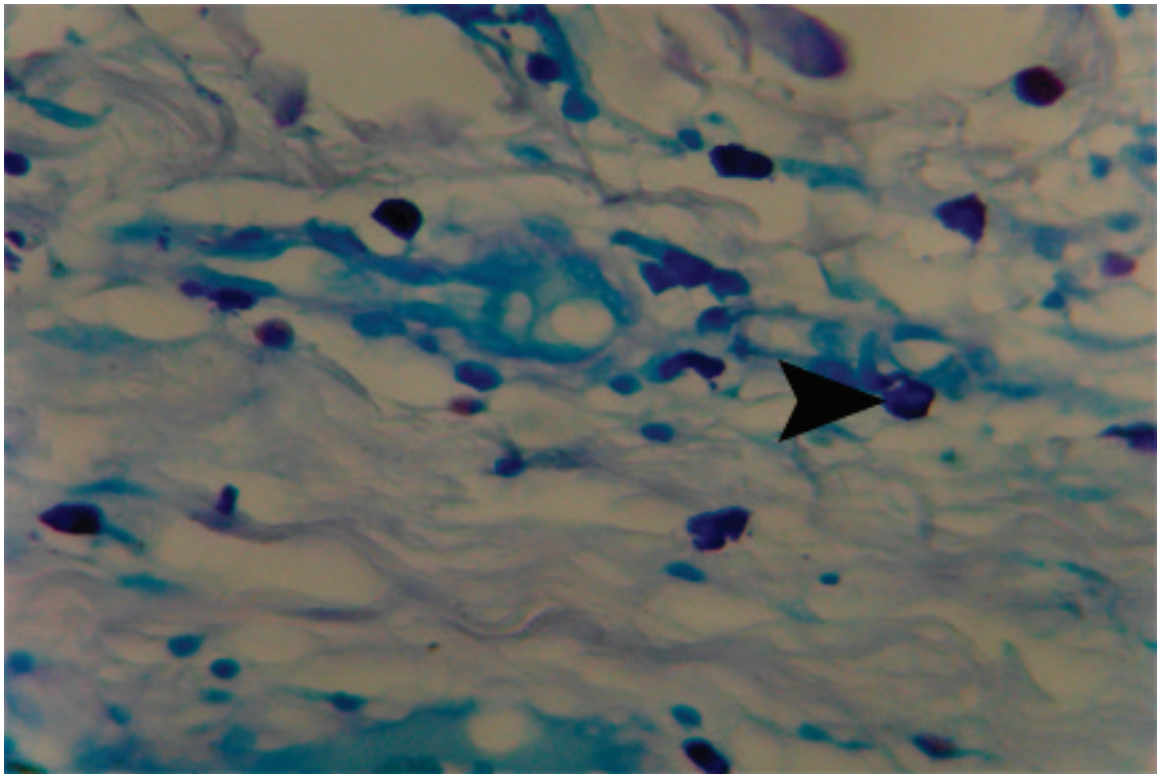

Figure 6. Microscopic features of round to oval (violet) mast cells (Arrow head, Giemsa stain X400).

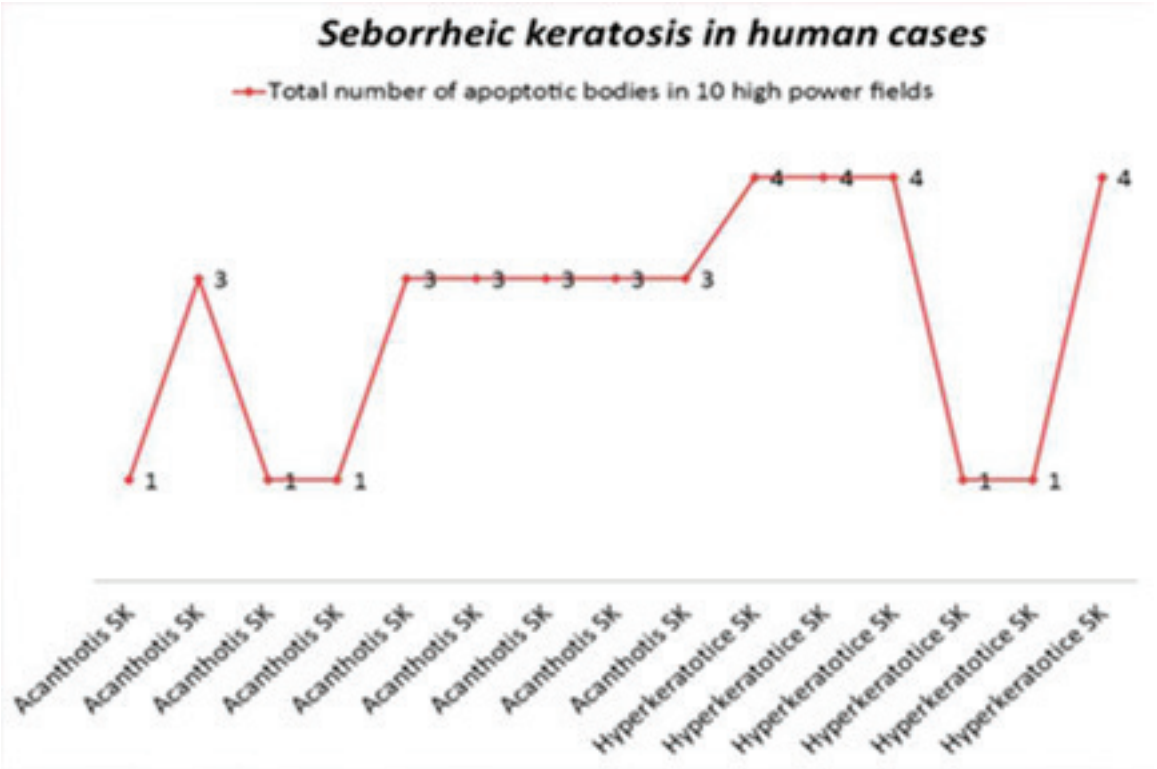

Figure 7. Line chart showing the total number of apoptotic bodies in human group.

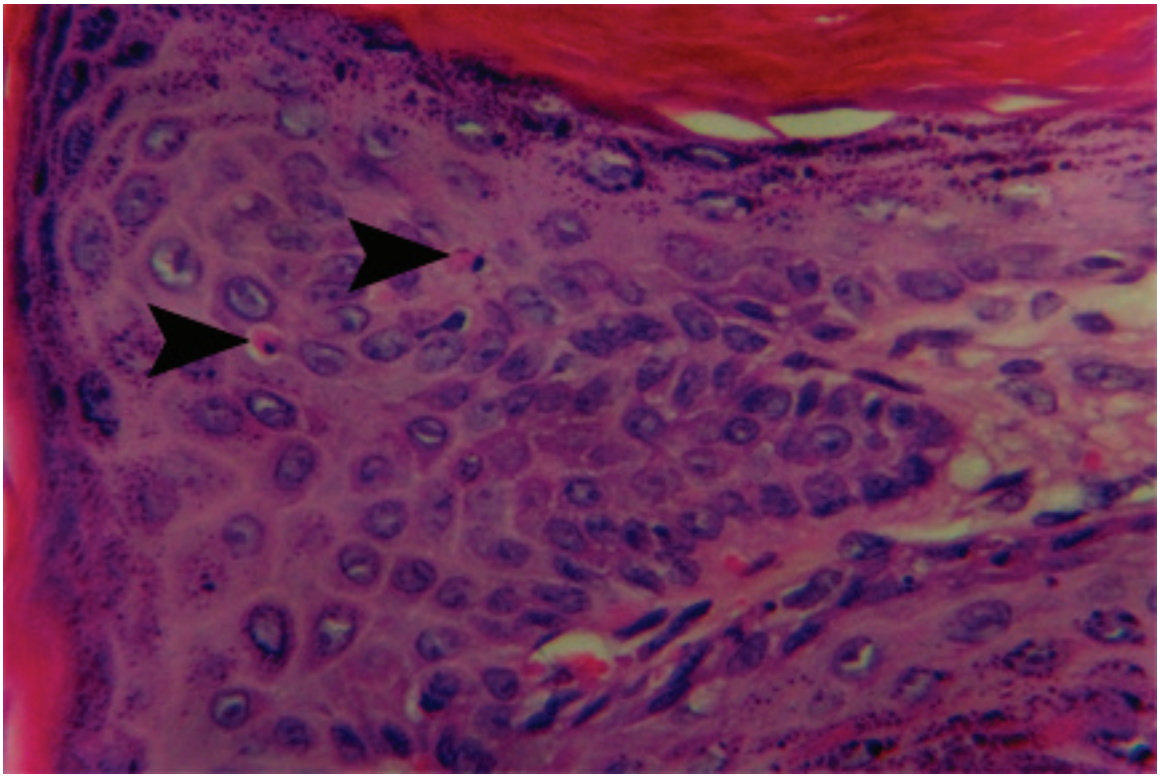

Figure 8. Microscopic features of apoptotic bodies (Arrow heads, H&E stain X400).

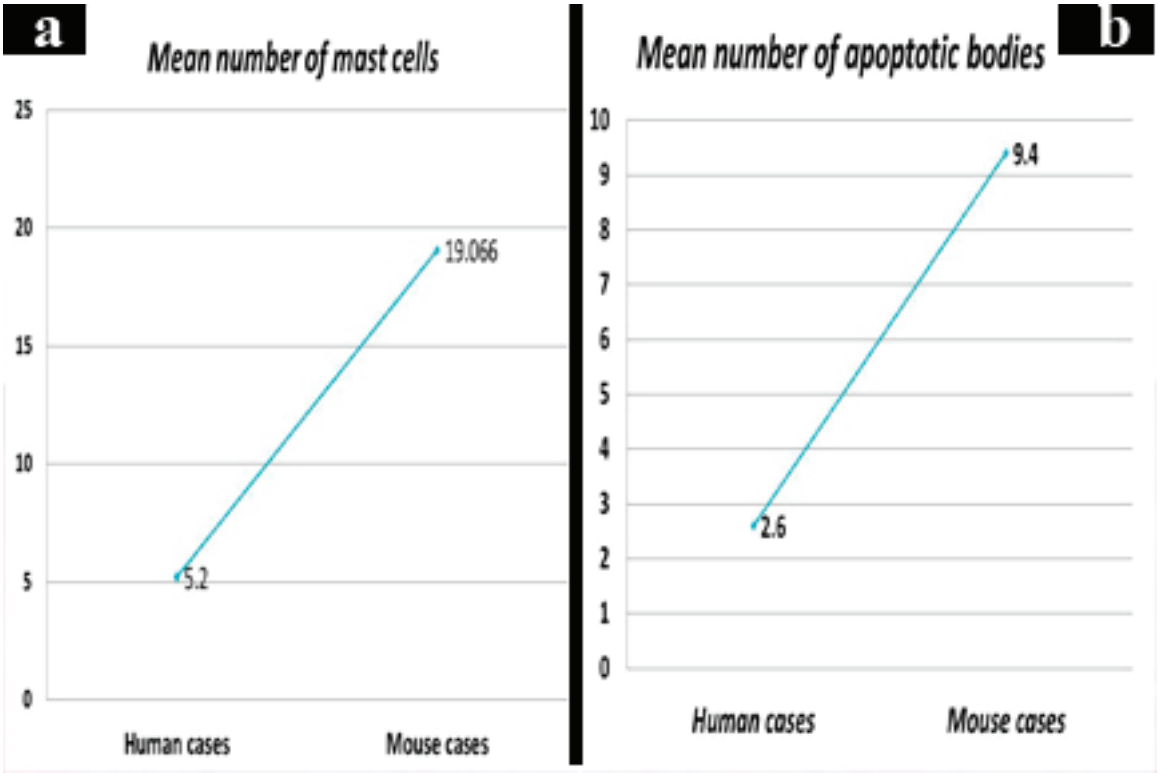

Figure 2. a; Line chart showing the mean number of mast cells of both groups (Human and Mouse), b; line chart showing the mean number of apoptotic bodies of both groups (Human and Mouse).

Table 1. Mast cells and apoptotic bodies between human and mouse groups using Pearson Correlation test.

	Mast cells in mouse group	Apoptotic bodies in mouse group	Mast cells in human group	Apoptotic bodies in human group
Mast cells in mouse group	1			
Apoptotic bodies in mouse group	0.110204	1		
Mast cells in human group	0.306618	0.246505	1	
Apoptotic bodies in human group	0.11423	0.024246	0.296296	1
Observed value	Datas that present in the above table			
Two-tailed p-value	< 0.01			
Alpha	0.05			

The closer the value is to 1or -1, the stronger the linear correlation.

Conclusion:

At the level of significance Alpha=0.050 the decision is to reject the null hypothesis of absence of correlation. In other words, the correlation is significant

DISCUSSION

According to previous studies the number of apoptotic bodies are variable in SK; moderate increase is observed in the rates of apoptosis in all varieties of SK compared to normal skin [23]. Rate of apoptosis in SK is not significantly different from normal skin [24]. Our work provided some evidences that the number of apoptotic bodies may differ according to the types of species (human and mice) which showed mild prevalence of apoptotic bodies in mice cases while for human apoptotic bodies were absent or decreased in number (in comparison to the normal skin histology for both species). This this finding was in disagreement with Bowen *et al*. and Balin, which they showed that apoptosis was moderately increased or its rate did not differ in comparison to normal skin [23-24].

In our result there was an increase in the number of mast cells in mice cases due to the effect of chronic UVB radiation. This was in agreement with Kligman and Murphy, Chacón-Salinas *et al*. [25-26], which documented that Chronic UVB irradiation increases the number of mast cells in the hairless mouse. As expected, we found that UVB exposure increased the number of mast cells in mice cases, whereas in human cases there was no increase in the number of mast cells; their shape was regularly rounded. With the major aim of the present study being to evaluate this strain susceptibility to UVB irradiation which led to an increase in the number of mast cells and indicate a strong relation between mast cells and chronic UVB irradiation in mice cases while for human cases may be not be related to UVB irradiation only, therefore mast cells slightly infiltrated into dermis. There are no previous studies that have mentioned increasing number of mast cells in SK in humans.

This result showed a very crucial correlation between mast cells and apoptotic bodies (Table 1). This correlation is in a reverse direction; so in mice cases the apoptotic bodies decreased while mast cells increased but in human cases there was a slight increase in mast cells infiltration and decrease in apoptosis. This was related to the effect of UVB radiation in mice cases. Up to our knowledge no previous studies have ever been published on this correlation and so this study may be the first to prove this.

In conclusion, VB triggers dermal mast cells infiltration. Mast cells may play a role in development of seborrheic keratosis and the number of apoptotic bodies in UVB induced cases are more than in sporadic cases.

REFERENCES

1. Naruke Y, Nakashima M, Suzuki K, Matsuu-Matsuyama M, Shichijo K, Kondo H, et al. Alteration of p53-binding protein 1 expression during skin carcinogenesis: Association with genomic instability. Cancer Science. 2008; 99: 946-51.

2. Saeed AK, Salmo N. Epidermal growth factor receptor expression in mice skin upon ultraviolet B exposure-seborrheic keratosis as a coincidental and unique finding. Adv Biomed Res. 2012; 1: 59.

3. Bhatia N, Demmer TA, Sharma AK, Elcheva I, Spiegelman VS. Role of β-TrCP ubiquitin ligase receptor in UVB mediated responses in skin. Arch Biochem Biophys. 2011; 508: 178-84.

4. Banerjee S. Seborrhoeic keratosis: Bilaterally symmetrical linear verrucous lesions in inguinal folds, an unusual presentation. Journal of Pakistan Association of Dermatologists. 2012; 22: 73-5.

5. Hafner C, Hartmann A, Van Oers A MM, Stoehr R, Zwarthoff EC, Hofstaedter F, et al. FGFR3 mutations in seborrheic keratoses are already present in flat lesions and associated with age and localization. Mod Pathol. 2007; 20: 895-903.

6. Ming M, Shea CR, Feng L, Soltani K, He YY. UVA Induces lesions resembling seborrheic keratoses in mice with keratinocyte-specific PTEN down-regulation. J Invest Dermatol. 2011; 131: 1583-6.

7. Lee ES, Whang MR and Kang WH. Absence of human papillomavirus DNA in nongenital seborrheic keratosis. J Korean Med Sci. 2001; 16: 619-22.

8. Bhuiyan ZH. Seborrheic keratosis: A case report. The Orion Medical Journal. 2007; 26: 441-2.

9. Storm CA, Elder DE. The skin. In: Rubin E, Reisner HM. Essentials of Rubin's Pathology. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2009. pp518.

10. Storm CA, Elder DE. The skin. In: Rubin R and Strayer DS. Rubin's Pathology: Clinicopathologic Foundations of Medicine. 5th ed. Baltimore: Lippincott Williams & Wilkins; 2008.pp1049.

11. Kwon OS, Hwang EJ, Bae JH. Seborrheic keratosis in the Korean males: Causative role of sunlight. Photodermatol Photoimmunol Photomed. 2003; 19: 73-80.

12. Wang JF, Wang B, Shehan JM, Sarma DP. Acantholytic Seborrheic Keratosis. The Internet Journal of Dermatology. 2008; 6: 1-4.

13. Kirkham N. Tumors and cysts of the epidermis. In: Elder DE, Elenitsas R, Johnson B, Murphy GF, Xu X. Lever's Histopathology of the Skin, 10th ed. India: Lippincott Williams &Wilikins; 2009. pp795-8

13. Murphy GF, Sellheyer K, Mihm MC. The skin. In: Kumar V, Abbas AK, Favsto N. Skin neoplasm. Robbins and Cotran Pathologic Basis of Disease. 7th ed. China: Elsevier Saunders; 2005. pp1230.

14. Rajabi P, Adibi N, Nematollahi P, Heidarpour M, Eftekhari M, Siadat AH. Bowenoid transformation in seborrheic keratosis: A retrospective analysis of 429 patients. J Res Med Sci. 2012; 17: 217-21.

15. Logie A, Dunois-Larde C, Rosty C. Activating mutations of the tyrosine kinase receptor FGFR3 are associated with benign skin tumors in mice and humans. Hum Mol Genet. 2005; 14: 1153-60.

13. Hassan SMA. The role of Acetyl salicylic acid on COX-2 expression in UVB- irradiated skin mouse utilizing immunohistochemistry. MSc thesis: University of Sulaimani; Iraq, 2011.

17. Kulms D, Schwarz T. Molecular mechanisms of UV-induced apoptosis. Photodermatology, Photoimmunology & Photomedicine. 2000; 16: 195-201.

18. Claerhout S, Laethem AV, Agostinis P, Garmyn M. Pathways involved in sunburn cell formation: Deregulation in skin cancer. Photochem Photobiol Sci. 2006; 5: 199-207.

19. Katiyar SK, Mantena SK, Meeran SM. Silymarin protects epidermal keratinocytes from ultraviolet radiation-induced apoptosis and DNA damage by nucleotide excision repair mechanism. Plos One. 2011; 6: 1-11.

20. Byrne SN, Limo´n-Flores AY, Ullrich SE. Mast cell migration from the skin to the draining lymph nodes upon ultraviolet irradiation represents a key step in the induction of immune suppression. J Immunol. 2008; 180: 4648-55.

21. Huang B, Lei Z, Zhang G, Dong L, Song C, Li B, et al. SCF-mediated mast cell infiltration and activation exacerbate the inflammation and immunosuppression in tumor microenvironment. Blood. 2008; 112: 1269-79.

23. Balin AK. Seborrheic keratosis, http://emedicine.medscape.com/article/1059477-overview, retrieved 2013.

24. Bowen AR, Hanks AN, Murphy KJ, Florell SR, Grossman D. Proliferation, apoptosis and survivin expression in keratinocytic neoplasms and hyperplasia. Am J Dermatopathol. 2004; 26: 177-81.

25. Kligman LH, Murphy GF. Ultraviolet B radiation increases hairless mouse mast cells in a dose-dependent manner and alters distribution of UV-induced mast cell growth factor. Photochem Photobiol. 1996; 63: 123-7.

26. Chacón-Salinas R, Limón-Flores AY, Chávez-Blanco AD, Gonzalez-Estrada A, Ullrich SE. Mast cell-derived IL-10 suppresses germinal center formation by affecting T follicular helper cell function. J Immunol. 2011; 186: 25-31.