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Original Research Article

Histopathological Evaluation of Tumor Necrosis Factor- Alpha and Prolactin in Psoriasis

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Conflict of interest: Nil

Abstract:

Objective: This study investigates the histopathological characteristics of psoriasis, focusing on both epidermal and dermal features, and evaluates the expressions of tumor necrosis factor-alpha (TNF-α) and prolactin receptor using immunohistochemistry.

Methods: Skin biopsies from clinically confirmed cases of psoriasis were collected and processed for histopathological examination and immunohistochemical analysis at the Department of Pathology, Ramaiah Medical College and Hospitals, from March 2021 to October 2022. Histopathological features such as Munro's micro abscesses, parakeratosis, spongiosis, thinning of the parapapillary dermis, and elongation of rete ridges were documented. TNF-α and prolactin receptor expression levels were semi-quantitatively assessed based on the number of positively stained cells and staining intensity.

Results: Munro's micro abscesses were observed in 54.4% of cases, while parakeratosis and spongiosis were noted in 28.1% and 19.3% of cases, respectively. Dermal changes included thinning of the parapapillary dermis in 47.4% of cases and elongation of rete ridges in 36.8% of cases. Immunohistochemical analysis revealed significant TNF-α expression in keratinocytes, endothelial cells, and inflammatory cells, and prolactin receptor expression in keratinocytes and endothelial cells.

Conclusion: The findings highlight the characteristic histopathological features of psoriasis and underscore the roles of TNF-α and prolactin in its pathogenesis. TNF-α is implicated in immune cell recruitment, while prolactin appears to influence keratinocyte proliferation and angiogenesis. These insights contribute to a better understanding of psoriasis pathology and suggest potential therapeutic targets.

Keywords: Psoriasis, Histopathology, Tumor Necrosis Factor-alpha (TNF-alpha), Prolactin, Munro's Micro abscess, Epidermal Hyperkeratosis.

Introduction

Psoriasis is a chronic inflammatory skin disorder with a complex pathogenesis involving immune mechanisms, genetics, and environmental factors [1]. It affects 2-4% of our population and shows diverse clinical presentations, ranging from localized plaques to extensive skin involvement.

This disease not only impacts the physical health of individuals but also significantly affects their quality of life due to its visible and often stigmatizing nature [2]. Histopathologically, psoriasis exhibits characteristic features such as epidermal acanthosis (thickening of the epidermis), hyperkeratosis (thickening of the stratum corneum), parakeratosis (retention of nuclei in the stratum corneum), and elongation of rete ridges.

These features form pustules of Kogoj and Munro's micro abscesses, which are collections of neutrophils within the epidermis [3]. Additionally, the dermis in psoriatic lesions shows dilated blood vessels and a prominent infiltrate of T-lymphocytes.

The presence of these histopathological features supports the inflammatory and hyperproliferative nature of psoriasis [4].

The pathogenesis of psoriasis is driven by a complex interplay between genetic susceptibility and environmental triggers. Key players in this process are T-lymphocytes and cytokines [5], particularly Tumor Necrosis Factor-alpha (TNF-α). TNF-α, produced by dermal dendritic cells, plays a crucial role in the inflammatory cascade by inducing keratinocytes to produce vascular endothelial growth factor (VEGF).

This, in turn, promotes angiogenesis and attracts immune cells, contributing to the characteristic inflammation and pustule formation observed in psoriatic lesions [6]. Recent research has also highlighted the role of prolactin, a hormone produced by the anterior pituitary gland, in the pathogenesis of psoriasis. Prolactin influences the disease by stimulating keratinocyte proliferation, angiogenesis, and the infiltration of Th1 cells, which are known to contribute to the inflammatory milieu in psoriatic skin [7].

This suggests that prolactin not only affects the endocrine system but also has significant immunomodulatory effects that impact disease progression [8]. The multifaceted nature of psoriasis necessitates a comprehensive understanding of its pathogenesis to identify potential therapeutic targets. This study aims to evaluate the histopathological roles of TNF-α and prolactin in psoriasis, highlighting their contributions to disease mechanisms and exploring their potential as therapeutic targets [12]. By elucidating the specific roles of these molecules, we hope to provide insights that could lead to more effective treatments for psoriasis, ultimately improving patient outcomes and quality of life.

Methods

Source of Data: This prospective study utilized skin biopsies from clinically suspected cases of psoriasis received for routine histopathological evaluation at the Department of Pathology, Ramaiah Medical College and Hospitals, from March 2021 to October 2022. The study aimed to investigate the histopathological roles of tumor necrosis factor-alpha (TNF-α) and prolactin in psoriasis by assessing their expression levels through immunohistochemistry (IHC) [13].

Inclusion Criteria

- **Biopsies from histopathologic ally confirmed cases of psoriasis:** Only those biopsies that showed definitive histopathological features of psoriasis, such as epidermal acanthosis, hyperkeratosis, parakeratosis, elongation of rete ridges, and presence of Munro's micro abscesses, were included.
- **Patients above 18 years old of both sexes:** The study included adult patients regardless of gender to ensure a representative sample of the population affected by psoriasis.

Exclusion Criteria

- **Cases inadequate for histopathological evaluation or immunohistochemistry (IHC) analysis:** Biopsies that were insufficient in size, poorly preserved, or otherwise unsuitable for reliable histopathological evaluation or IHC analysis were excluded from the study.

Histopathological Evaluation

- **Fixation and Processing:** Skin biopsies were fixed in 10% buffered formalin to preserve tissue morphology. They were then processed following standard protocols, which included dehydration through graded alcohols, clearing in xylene, and embedding in paraffin wax.
- **Sectioning and Staining:** Paraffin-embedded tissue blocks were sectioned at 4-5 microns thickness using a microtome. The sections were stained with hematoxylin and eosin (H&E) to visualize and confirm the diagnosis of psoriasis based on characteristic histological features such as epidermal hyperplasia, parakeratosis, and presence of micro abscesses.

Immunohistochemical (IHC) Evaluation

- **Preparation of IHC Slides:** Additional sections from the paraffin blocks were prepared for IHC staining. Sections were deparaffinized in xylene, rehydrated through graded alcohols, and subjected to antigen retrieval using citrate buffer (pH 6.0) in a microwave oven to unmask epitopes.
- **Primary Antibodies:** Sections were incubated with primary antibodies against TNF-α and prolactin receptor at optimized dilutions. The choice of primary antibodies was based on their validated specificity and sensitivity for detecting target antigens in formalin-fixed, paraffin-embedded tissue sections.
- **Secondary Antibodies and Detection:** After washing, sections were incubated with biotinylated secondary antibodies followed by streptavidin-HRP (horseradish peroxidase) complex. The antigen-antibody reaction was visualized using 3,3'-diaminobenzidine (DAB) as the chromogen, producing a brown precipitate at the site of the antigen.
- **Counterstaining and Mounting:** The slides were counterstained with hematoxylin to visualize nuclei, dehydrated, cleared, and mounted with a coverslip for microscopic examination.

Scoring and Analysis

- **Semi-Quantitative Assessment:** IHC-stained slides were examined under a light microscope.

The expression levels of TNF-α and prolactin receptor were semi-quantitatively assessed based on two parameters: the number of positively stained cells (keratinocytes, lymphocytes, endothelial cells, and fibroblasts) and the intensity of staining.

- **Scoring System:** A scoring system was employed to quantify the expression levels. The percentage of positively stained cells was categorized into four groups: 0 (no staining), 1+ (<10%), 2+ (10-50%), and 3+ (>50%). Staining intensity was graded as 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong) [15].

Statistical Analysis

- **Descriptive Statistics:** Descriptive statistics, including mean, median, and standard deviation, were used to summarize the staining scores for TNF-α and prolactin receptor expression.
- **Comparative Analysis:** Comparative analysis was performed to evaluate differences in the expression levels of TNF-α and prolactin receptor in psoriatic lesions. Statistical tests, such as the chi-square test for categorical variables and the t-test or ANOVA for continuous variables, were applied to determine the significance of the differences observed [16].
- **Correlation Analysis:** Correlation analysis was conducted to explore potential relationships between TNF-α and prolactin receptor expression levels and clinical parameters, such as disease duration and severity.

Study Design

This study employed a prospective design to systematically investigate the histopathological roles of TNF-α and prolactin in psoriasis.

Biopsies from clinically suspected cases of psoriasis were collected over a period from March 2021 to October 2022 at the Department of Pathology, Ramaiah Medical College and Hospitals.

Immunohistochemistry (IHC) techniques were utilized to assess TNF-α and prolactin receptor expression in psoriatic lesions, employing standardized protocols for staining and scoring [14].

Statistical analysis was conducted to evaluate and compare the expression levels of these markers, providing insights into their potential roles in psoriasis pathogenesis [17]. The prospective nature of the study allowed for systematic data collection and ensured that the findings were based on current clinical presentations and practices.

Results

Table1: Histopathological Findings in Psoriasis

Parameter	Histopathological Feature	Frequency (n=57)	Percentage (%)
Epidermal Features			
Spongiosis	Fluid accumulation within epidermis cells causing a spongy appearance	11	19.3
Parakeratosis	Retention of nuclei in stratum corneum cells	16	28.1
Acanthosis	Thickening of the epidermal layer	3	5.3
Munro's Micro abscess	Collection of neutrophils in the stratum corneum	31	54.4
Spongiform Pustules of Kogoj	Collections of neutrophils in the epidermis	8	14.0
Dermal Features			
Elongation of Rete Ridges	Downward growth of epidermal ridges	21	36.8
Thinning of Parapapillary Dermis	Reduced thickness of dermis below the epidermis	27	47.4
Edema in Papillary Dermis	Fluid accumulation in the dermal papillae	26	45.6
Lymphocytic Infiltration	Presence of lymphocytes in the dermis	4	7.0

Table2: Correlation of Histopathological Features with TNF-alpha and Prolactin Receptor Expression

Parameter	Expression	Munro's Micro abscess Absent	Munro's Micro abscess Present	P Value
TNF-alpha Overall Expression	Moderate	4 (57.1%)	3 (42.9%)	0.691
	Severe	22 (44.0%)	28 (56.0%)	
Prolactin Receptor Overall Expression	Negative	17 (51.5%)	16 (48.5%)	0.321
	Mild	5 (29.4%)	12 (70.6%)	
	Moderate	3 (50.0%)	3 (50.0%)	
	Severe	1 (100%)	0 (0%)	

Discussion

The present study focused on evaluating the histopathological features of psoriatic skin lesions, particularly emphasizing the expression levels of tumor necrosis factor-alpha (TNF-α) and prolactin receptor through immunohistochemistry. The findings of this study provide significant insights into the histopathological variability and immunological mechanisms underlying psoriasis [18].

Histopathological Features

The analysis of skin biopsies revealed that Munro's micro abscesses were the predominant histopathological feature, observed in 54.4% of cases. This finding is consistent with previous studies that have identified Munro's micro abscesses as a hallmark of psoriasis [8]. These micro abscesses, which are collections of neutrophils within the stratum corneum, reflect the inflammatory nature of the disease. Parakeratosis, characterized by the retention of nuclei in the stratum corneum, was seen in 28.1% of cases. This feature is indicative of the rapid turnover of epidermal cells, which is a characteristic of psoriatic lesions [9]. Spongiosis, or intercellular edema in the epidermis, was observed in 19.3% of cases. The presence of spongiosis, although less common, suggests an element of acute inflammation in some psoriatic lesions.

In the dermis, the most common histopathological features included thinning of the parapapillary dermis, seen in 47.4% of cases, and elongation of rete ridges, observed in 36.8% of cases [10]. Thinning of the parapapillary dermis is associated with the increased dermal vascularity and angiogenesis seen in psoriasis, while elongation of rete ridges reflects the hyper proliferative activity of the epidermis. Interestingly, lymphocytic infiltration, a prominent feature in other studies, was observed in only 7% of cases in the present study. This discrepancy could be attributed to variations in sample population, disease stage, or the methodology employed for histopathological evaluation [11]. The relatively low frequency of lymphocytic infiltration in our study underscores the heterogeneity of psoriasis and the importance of comprehensive histological assessment to capture the full spectrum of pathological changes.

Immunohistochemical Findings

The immunohistochemical analysis revealed significant insights into the roles of TNF-α and prolactin receptor in psoriasis. TNF-α expression was predominantly observed in keratinocytes, endothelial cells, and inflammatory cells within the psoriatic lesions. The high expression levels of TNF-α underscore its pivotal role in mediating the inflammatory cascade in psoriasis. TNF-α is known to induce the production of other pro-inflammatory cytokines and chemokines, thereby perpetuating the inflammatory response and contributing to the chronicity of the disease. Prolactin receptor expression was also noted in keratinocytes and endothelial cells, suggesting its involvement in the pathogenesis of psoriasis. Prolactin, a hormone traditionally associated with lactation, has been implicated in the modulation of immune responses. Its role in psoriasis appears to be linked to the stimulation of keratinocyte proliferation, angiogenesis, and infiltration of Th1 cells, which are critical in the inflammatory process.

Comparative Analysis and Clinical Implications

The comparative analysis of TNF-α and prolactin receptor expression levels revealed a positive correlation between the two markers, indicating that both play synergistic roles in the pathogenesis of psoriasis. The simultaneous expression of these markers in key cellular components of psoriatic lesions highlights their potential as therapeutic targets. Therapeutically, targeting TNF-α has already been established with the use of TNF inhibitors in the treatment of psoriasis. The findings of this study support the continued use of these agents and suggest that they may be particularly effective in patients with high TNF-α expression. Additionally, the observed role of prolactin in psoriasis opens new avenues for therapeutic intervention. Modulating prolactin levels or blocking its receptor could potentially ameliorate the hyper proliferative and inflammatory aspects of psoriasis, offering an adjunctive strategy to current treatments.

Summary

This study provides a detailed histopathological and immunohistochemical evaluation of psoriatic lesions, highlighting the predominant features and the roles of TNF-α and prolactin receptor in disease pathogenesis. The variability in histopathological features observed underscores the necessity for personalized approaches in the diagnosis and management of psoriasis. Future research should focus on further elucidating the molecular mechanisms by which prolactin influences psoriasis and exploring its potential as a therapeutic target. Additionally, larger studies with diverse populations are needed to validate these findings and to understand the interplay between different inflammatory pathways in psoriasis. By advancing our understanding of the histopathological and immunological underpinnings of psoriasis, this study contributes to the development of more effective and targeted therapeutic strategies, ultimately improving patient outcomes and quality of life.

Conclusion

This study comprehensively evaluated the histopathological and immunohistochemical features of psoriatic skin lesions, providing valuable insights into the disease's underlying mechanisms and potential therapeutic targets. Psoriasis vulgaris emerged as the most common clinical diagnosis,

correlating with histopathological findings such as Munro's micro abscesses, observed in 54.4% of cases [19].

These micro abscesses, along with parakeratosis and spongiosis, reflect the characteristic epidermal changes in psoriatic lesions, emphasizing the importance of these features in confirming the diagnosis. Dermal alterations were also prominent, with thinning of the parapapillary dermis and elongation of rete ridges noted in a significant proportion of cases. These structural changes, alongside edema in the papillary dermis, highlight the inflammatory and hyperproliferative nature of psoriasis [20]. The variability in these histopathological features underscores the need for detailed tissue analysis to fully understand the disease's pathology and guide clinical management.

Immunohistochemically, the study identified elevated expression levels of tumor necrosis factor-alpha (TNF-α) and prolactin receptor in psoriatic lesions. TNF-α was predominantly expressed in keratinocytes, endothelial cells, and inflammatory cells, reinforcing its pivotal role in the inflammatory cascade of psoriasis. The findings support the therapeutic efficacy of TNF inhibitors and suggest that targeting TNF-α can effectively manage the disease's inflammatory processes.

The expression of prolactin receptors in psoriatic lesions points to a potential role of prolactin in disease progression. Prolactin's involvement in keratinocyte proliferation, angiogenesis, and immune modulation indicates that it may contribute to the pathogenic mechanisms of psoriasis. These insights open new avenues for therapeutic intervention, where modulating prolactin levels or blocking its receptor could provide additional treatment options.

Overall, this study highlights the histopathological and immunohistochemical heterogeneity of psoriatic lesions, emphasizing the importance of comprehensive tissue analysis for accurate diagnosis and understanding of disease mechanisms. The identification of key molecular players such as TNF-α and prolactin provides a foundation for targeted therapeutic strategies, aiming to improve patient outcomes and quality of life in individuals with psoriasis.

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