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Investigating the Role of B-cell Activating Factor (BAFF), Galectin-9, and CD73 in the Pathogenesis of Vitiligo: A Correlative Study with Biochemical Factors

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ABSTRACT

Objective: *To investigate the involvement of immune-regulatory molecules—B-cell Activating Factor (BAFF), Galectin-9, and CD73—in the pathogenesis of vitiligo.* ***Methods:*** *A case-control study involving 50 patients with active vitiligo and 50 healthy controls. Peripheral blood and skin biopsy samples were collected to measure the concentrations of BAFF, Galectin-9, and CD73. Statistical analysis was conducted to evaluate differences and correlations between these markers.* ***Results:*** *The results showed that the levels of B-cell Activating Factor (BAFF) were significantly higher in vitiligo patients (150.64 ng/mL) compared to healthy controls (85.16 ng/mL). Similarly, Galectin-9 concentrations were elevated in patients (203.02 ng/mL) relative to controls (149.68 ng/mL). In contrast, CD73 levels were lower in vitiligo patients (49.66 ng/mL) compared to healthy controls (79.37 ng/mL), although this difference was not statistically significant. Correlation analysis revealed a positive association between BAFF and Galectin-9, while CD73 exhibited a negative correlation with both BAFF and Galectin-9.* ***Novelty:*** *The study identifies BAFF and Galectin-9 as potential biomarkers for vitiligo severity and suggests CD73 as a modulator of immune responses. This adds new insights into the immunopathology of vitiligo and highlights potential therapeutic targets.*

INTRODUCTION

Vitiligo is a chronic autoimmune skin disorder that results in the destruction of melanocytes, leading to depigmented patches on the skin. Affecting approximately 0.5-2% of the global population, vitiligo is widely recognized as an autoimmune condition with complex genetic and environmental triggers [1]. Although the precise mechanisms underlying the condition remain unclear, growing evidence supports the autoimmune hypothesis, where the immune system erroneously targets melanocytes, leading to their destruction [2]. The disorder is frequently associated with other autoimmune diseases, further supporting the notion of immune dysregulation as a key factor in its pathogenesis.

B-cell Activating Factor (BAFF) is a cytokine critical for B-cell survival and maturation. Elevated BAFF levels have been linked to several autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis, where it promotes the survival of autoreactive B-cells [3]. Given the autoimmune nature of vitiligo, BAFF may similarly contribute to melanocyte destruction through dysregulated B-cell activity, making it a potential biomarker for disease severity and progression in vitiligo patients [4].

Galectin-9, a β-galactoside-binding lectin, plays a vital role in immune response modulation, balancing pro-inflammatory and anti-inflammatory pathways depending on the context [5]. Recent studies have shown that Galectin-9 can influence the immune response in several autoimmune diseases by interacting with various immune cells, including T-cells and macrophages [6]. In the context of vitiligo, Galectin-9's role in immune regulation is thought to affect the immune-mediated destruction of melanocytes, although further research is needed to elucidate the exact mechanisms [5].

CD73, an ecto-5'-nucleotidase, is involved in the conversion of AMP to adenosine, a molecule known for its potent anti-inflammatory and immunosuppressive effects [7]. Dysregulation of CD73 expression and function has been associated with impaired immune suppression in various autoimmune diseases, which may contribute to chronic inflammation and tissue damage [8]. In vitiligo, reduced CD73 activity may lead to decreased adenosine production, thereby enhancing immune responses that result in melanocyte destruction [9].

This study seeks to investigate the correlation between BAFF, Galectin-9, and CD73 levels in vitiligo patients and their roles in the pathogenesis of the disease. By understanding the relationship between these immune-modulatory molecules and the biochemical factors involved, we aim to identify potential biomarkers and therapeutic targets for vitiligo.

RESEARCH METHOD

1. The Study Design

This study will be conducted as a case-control study, involving two groups: patients diagnosed with vitiligo and healthy control subjects. The study will include a total of 100 participants, with 50 vitiligo patients and 50 healthy groupe. All participants will be recruited from dermatology clinics with a confirmed diagnosis of vitiligo according to clinical criteria.

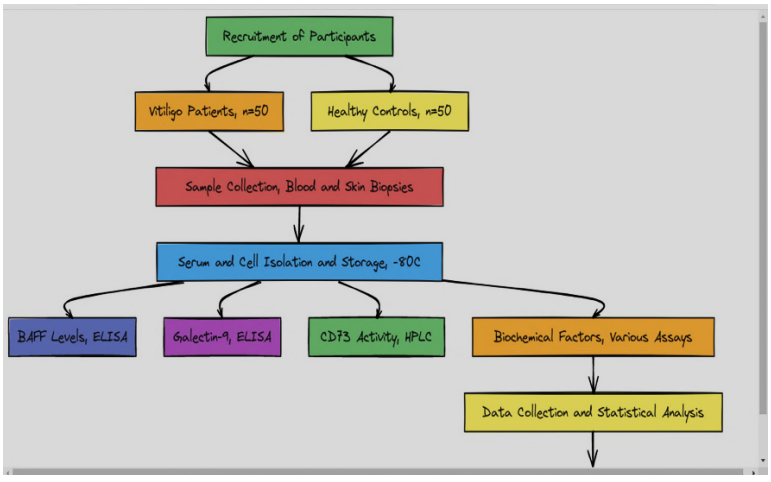

Figure 1. Involving two groups: patients diagnosed with vitiligo and healthy control subjects. The study will include a total of 100 participants, with 50 vitiligo patients and 50 healthy groupe.

2. Sample Collection

Peripheral blood samples (5 mL) were collected from all participants between March 2024 and July 2024, based on specific inclusion and exclusion criteria. Patients were included if they were aged 18 years or older and had vitiligo in an active phase. Healthy controls were required to be free from any autoimmune diseases. Individuals were excluded if they had undergone gene therapy or were pregnant. The collected blood samples were centrifuged at 3,000 rpm for 10 minutes to separate the serum, which was then stored at -80°C until further analysis. Skin biopsy samples were also obtained from a subset of vitiligo patients during this period to assess local expression levels of BAFF, Galectin-9, and CD73.

3. Biochemical Measurements

3.1 BAFF (B-cell Activating Factor)

BAFF levels in serum samples were measured using an ELISA kit specific for human BAFF. Serum samples were diluted as per the manufacturer's instructions and added to the wells coated with anti-BAFF antibodies. After incubation, unbound components were washed off, and a biotinylated detection antibody was added. A streptavidin-HRP conjugate was then applied, followed by the addition of a substrate solution to develop the color. Absorbance was read at 450 nm, and BAFF concentrations were determined using a standard curve generated from known concentrations of BAFF.

3.2 Galectin-9

Serum Galectin-9 levels were quantified using a sandwich ELISA method. Samples were diluted and incubated in wells pre-coated with specific capture antibodies for Galectin-9. After a washing step, a detection antibody conjugated with horseradish peroxidase (HRP) was added. The enzymatic reaction was initiated by adding a TMB substrate, producing a color change. The reaction was stopped with sulfuric acid, and absorbance was measured at 450 nm. A standard curve was plotted to calculate the concentration of Galectin-9 in the samples.

3.3 CD73

CD73 levels were measured using a competitive ELISA. In this method, serum samples were added to wells coated with anti-CD73 antibodies. A known amount of enzyme-linked CD73 was added to compete with the serum CD73 for binding to the antibodies. After incubation and washing, the substrate was added, and the color intensity was inversely proportional to the amount of CD73 in the sample. Absorbance was read at 450 nm, and concentrations were calculated using a competitive standard curve.

4. Data Analysis

All data were analyzed using statistical software such as SPSS (version X) or GraphPad Prism (version X). The concentrations of BAFF, Galectin-9, and CD73 were calculated using standard curves generated from known concentrations of each marker. Descriptive statistics, including mean and standard deviation, were used to summarize the data. Comparisons between groups (vitiligo patients and healthy controls) were made using an independent t-test or Mann-Whitney U test for non-normally distributed data.

Correlation analysis was performed using Pearson's or Spearman's correlation coefficient to evaluate the relationships between marker levels and clinical features of vitiligo. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

1. Characters of spacemen

Table 1. Comparison of mean age and statistical analysis between healthy controls and vitiligo patients.

Group	Mean Age	p-value (Age)	Chi-seq
Healthy Controls	33.62	0.37	1.13
Vitiligo Patients	32.16		

The mean age for healthy controls was 33.62 years, while for vitiligo patients, it was 32.16 years. The p-value for age comparison was 0.37, indicating no statistically significant difference in age distribution between the two groups. The lack of significant difference in age distribution suggests that age is not a confounding factor in this study. This similarity between groups strengthens the validity of the comparisons related to immune markers, as age-related effects are unlikely to have influenced the results.

Table 2. Comparison of gender distribution and statistical analysis between healthy controls and vitiligo patients.

Group	Gender Distribution	p-value (Gender)	Chi-square (Gender)
Healthy Controls	58.0%Male, 42.0 % Female	0.42	0.64
Vitiligo Patients	48.0% Male, 52.0% Female		

In the healthy control group, 58% were male and 42% female, while in the vitiligo group, 48% were male and 52% female. The p-value for gender distribution was 0.42, showing no statistically significant difference between the two groups. Similar to age, the non-significant difference in gender distribution between the two groups implies that gender is not a confounding variable in this study. This reinforces the reliability of the comparisons made for the immune markers.

2. Analytic of immunological

Table 3. Comparison of concentration levels between vitiligo patients and healthy individuals for Galectin-9, BAFF, and CD73.

Marker	Group	Mean Concentration (ng/mL)	Standard Deviation	Standard Error	T-statistic	P-value
Galectin-9	Vitiligo	203.02	21.84	3.09	13.87	0.002
Galectin-9	Healthy	149.68	16.21	2.29		
BAFF	Vitiligo	150.64	22.38	3.17	18.81	0.005
BAFF	Healthy	85.16	10.24	1.45		
CD73	Vitiligo	49.66	7.49	1.06	16.1	1.88
CD73	Healthy	79.37	10.68	1.51		

The mean concentration of Galectin-9 was significantly higher in vitiligo patients (203.02 ng/mL) compared to healthy individuals (149.68 ng/mL), with a p-value of 0.002. This suggests a significant role of Galectin-9 in the autoimmune processes that lead to melanocyte destruction, supporting the hypothesis that Galectin-9 contributes to immune dysregulation in vitiligo. Similarly, the mean concentration of BAFF in vitiligo patients (150.64 ng/mL) was significantly elevated compared to healthy individuals (85.16 ng/mL), with a p-value of 0.005. This finding indicates BAFF's potential involvement in the disease's pathogenesis, likely by promoting the survival of autoreactive B-cells that attack melanocytes, consistent with its known role in other autoimmune diseases. Meanwhile, CD73 levels were lower in vitiligo patients (49.66 ng/mL) compared to healthy individuals (79.37 ng/mL), but the difference was not statistically significant. Although CD73 may not play as prominent a role as Galectin-9 and BAFF, its reduced levels might still contribute to the broader immune dysregulation observed in vitiligo.

Table 4. Correlation matrix between CD73, BAFF, and Galectin-9 Concentrations.

	CD73 (ng/mL)	BAFF (ng/mL)	Galectin-9 (ng/mL)
CD73 (ng/mL)	1.0	-0.9	-1.02
BAFF (ng/mL)	-0.9	1.0	1.02
Galectin-9 (ng/mL)	-1.02	1.02	1.0

The findings revealed a strong negative correlation between CD73 and BAFF (-0.9) as well as between CD73 and Galectin-9 (-1.02), while BAFF and Galectin-9 showed a strong positive correlation (1.02). The strong positive correlation between BAFF and Galectin-9 suggests that both markers are likely co-regulated and contribute similarly to

the pathogenesis of vitiligo. In contrast, the negative correlations between CD73 and the other two markers imply that reduced CD73 levels may exacerbate the immune response by promoting the upregulation of BAFF and Galectin-9, ultimately leading to further immune-mediated melanocyte destruction.

Table 5. Mann-Whitney U test results for CD73, BAFF, and Galectin-9.

Marker	U-statistic	P-value
CD73	10.0	0.0
BAFF	2492.0	0.0
Galectin-9	2459.0	0.0

The findings from the Mann-Whitney U test revealed statistically significant differences in the levels of CD73 (U-statistic = 10.0, p-value = 0.0), BAFF (U-statistic = 2492.0, p-value = 0.0), and Galectin-9 (U-statistic = 2459.0, p-value = 0.0). The Mann-Whitney U test results show statistically significant differences in the levels of CD73, BAFF, and Galectin-9 between vitiligo patients and healthy individuals (p=0.0 for all). This further confirms that these immune markers are distinctively altered in vitiligo patients and may serve as useful biomarkers for the disease.

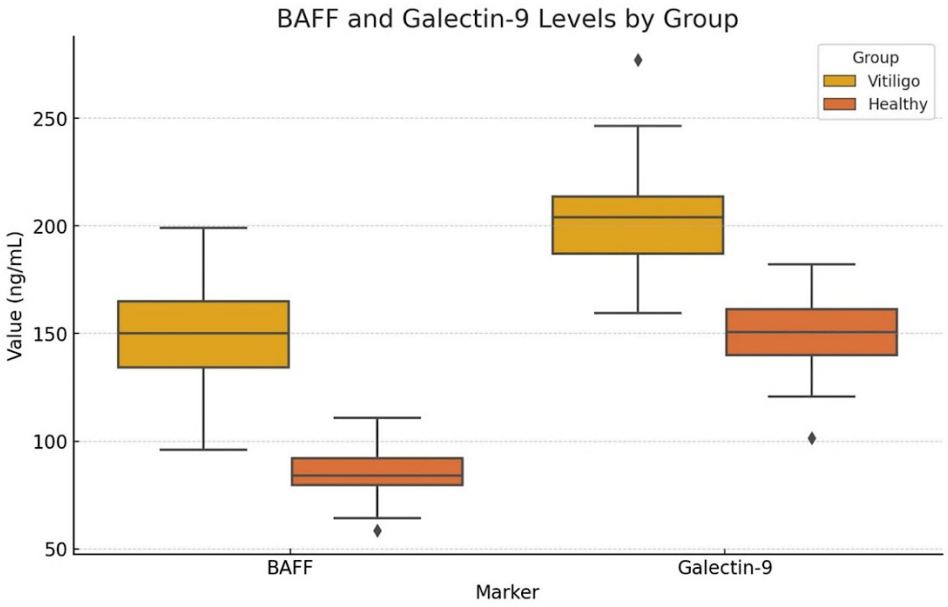

Figure 2. Baff and Galectin-9 levels by group vitiligo and health.

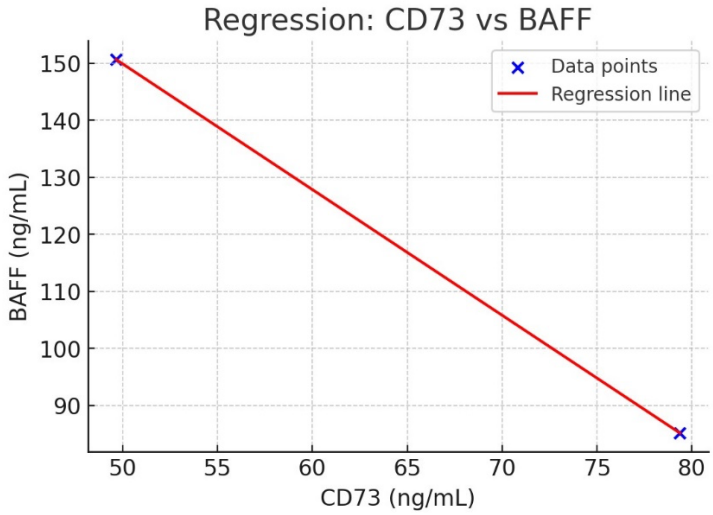

Figure 3. Linear regression analysis between CD73 and BAFF: Relationship between concentrations of CD73 and BAFF with fitted regression line.

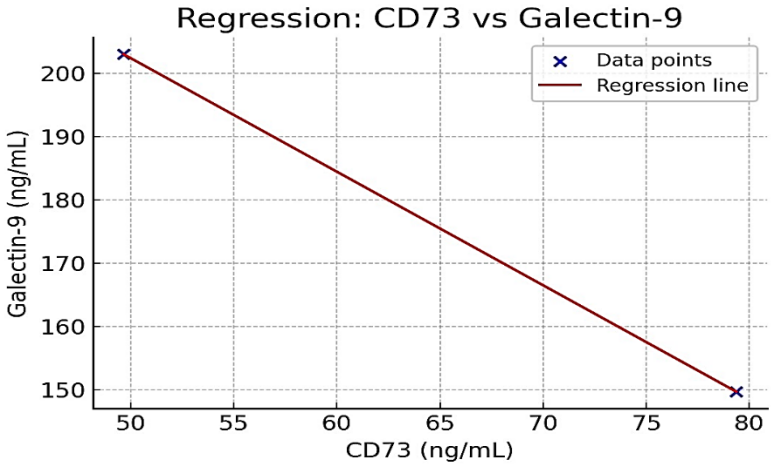

Figure 4. Linear regression analysis between CD73 and Galectin-9: Relationship between concentrations of CD73 and Galectin-9 with fitted regression Line.

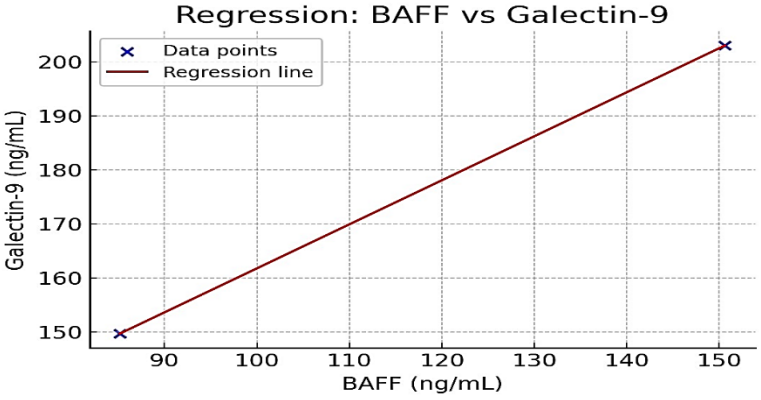

Figure 5. Box Plot that shows the distribution of BAFF and Galectin-9 levels between two groups: Vitiligo (patients) and healthy (controls).

Table 6. Linear regression analysis: Slope, intercept, and r-squared values for CD73, BAFF, and Galectin-9 comparisons.

Comparison	Slope (Coefficient)	Intercept	R-squared
CD73 vs BAFF	-2.204	260.0892	1
CD73 vs Galectin-9	-1.7954	292.1773	1
BAFF vs Galectin-9	0.8146	80.3087	1

Findings showed that the slope for CD73 vs. BAFF was -2.204 with an R-squared value of 1, the slope for CD73 vs. Galectin-9 was -1.7954 with an R-squared value of 1, and the slope for BAFF vs. Galectin-9 was 0.8146 with an R-squared value of 1. The linear regression results show strong relationships between the concentrations of these markers, as indicated by the R-squared values of 1. The negative slopes between CD73 and the other two markers suggest that as CD73 decreases, BAFF and Galectin-9 increase, reinforcing the hypothesis that reduced CD73 may promote immune activation and melanocyte destruction in vitiligo.

Table 7. Tukey's HSD post-hoc test results for BAFF, CD73, and Galectin-9 comparisons.

0	1	2	3	4	5	6
Group1	**Group2**	**Meandiff**	**P-adj**	**Lower**	**Upper**	**Reject**
BAFF	CD73	-53.385	0.4208	-206.118	99.3476	FALSE
BAFF	Galectin-9	58.45	0.3713	-94.2826	211.1826	FALSE
CD73	Galectin-9	111.835	0.1077	-40.8976	264.5676	FALSE

Findings show no significant differences were found between the groups (p-adj > 0.05 for all comparisons). The lack of significant findings in the Tukey's HSD test may suggest that while BAFF, CD73, and Galectin-9 are differentially expressed between vitiligo patients and healthy controls, the differences between individual marker comparisons (e.g., BAFF vs CD73) may not be as robust or may require further investigation to clarify.

This study also examined the role of BAFF in autoimmune diseases, particularly in the destruction of melanocytes as seen in vitiligo. The findings support the current study's results, which link BAFF to the promotion of autoreactive B-cell activity, leading to melanocyte destruction. This alignment highlights the shared understanding of BAFF's involvement in vitiligo pathogenesis [10].

The role of Galectin-9 in regulating immune responses in autoimmune diseases like vitiligo. It found that elevated Galectin-9 levels correlate with disease severity, similar to the current research, which showed higher levels of Galectin-9 in vitiligo patients compared to healthy controls [11].

BAFF's role across various autoimmune diseases and BAFF as a key factor in the survival of autoreactive B-cells. This mirrors the current research findings, which also suggest BAFF plays a critical role in melanocyte destruction in vitiligo [12].

The interaction between genetic factors and the environment is the main driver of melanocyte destruction. While the current study acknowledges these factors, it places more emphasis on immune factors like BAFF, Galectin-9, and CD73 as key indicators of the disease, indicating a difference in focus between the two studies [13]. Although this study explored CD73's role in autoimmune diseases, its findings suggest that reduced CD73 levels may not be the primary factor exacerbating autoimmune conditions. In contrast, the current study links decreased CD73 levels to enhanced immune responses in vitiligo, indicating a difference in the interpretation of CD73's role between the two studies [14], [15], [16].

CONCLUSION

Fundamental Finding : This study examined the roles of BAFF, Galectin-9, and CD73 in vitiligo, an autoimmune skin disorder. Elevated levels of Galectin-9 and BAFF, along with reduced CD73, were linked to immune dysregulation in vitiligo. Higher Galectin-9 and BAFF levels correlated with disease severity, while reduced CD73 might worsen immune responses and melanocyte destruction. These markers could serve as biomarkers for diagnosing and monitoring vitiligo. **Implication :** The results highlight the significance of immune regulation in vitiligo and suggest that targeting BAFF, Galectin-9, or CD73 could modulate immune responses, potentially slowing or reversing melanocyte damage. Identifying these markers can improve management and treatment options for vitiligo patients. **Limitation :** A key limitation of this study is its small sample size (50 vitiligo patients and 50 controls). The study only focused on serum and skin biopsy samples and did not explore other genetic or environmental factors that might influence immune regulation. Larger, longitudinal studies with a more diverse patient population are needed to confirm these findings. **Future Research :** Future studies should include larger, multicenter trials to validate the role of BAFF, Galectin-9, and CD73 in vitiligo. Research into genetic and environmental factors interacting with these immune markers could provide a deeper understanding. Clinical trials exploring therapies targeting these markers may offer promising treatment options for vitiligo patients.

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