



Hematological and Immunological Effects of Adipose Tissue-Derived Mesenchymal Stem Cells in Experimental Hashimoto's Thyroiditis in Wistar Rats

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Abstract

Introduction: Hashimoto's thyroiditis (HT) is an autoimmune disorder characterized by lymphocytic infiltration and progressive destruction of thyroid tissue, resulting in hypothyroidism. Current management primarily relies on hormone replacement and does not target the underlying autoimmune process. This study aimed to evaluate the therapeutic effects of adipose tissue-derived mesenchymal stem cells (ADMSCs) on hematological and immunological alterations in experimentally induced HT in Wistar rats.

Materials and Methods: Forty female Wistar rats were randomly assigned to four groups (n = 10 per group): an HT group, an HT group treated with ADMSCs, an ADMSC-only group, and a healthy control group. HT was induced by subcutaneous administration of porcine thyroglobulin emulsified with Freund's adjuvants. Disease induction was confirmed by measuring anti-thyroglobulin antibody (Anti-Tg Ab) and thyroid hormone levels. Hematological indices, thyroid function tests, and inflammatory and immunoregulatory biomarkers were assessed. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant.

Results: The HT group exhibited significantly higher Anti-Tg Ab and thyroid-stimulating hormone (TSH) levels, lower free triiodothyronine (FT3) and free thyroxine (FT4) concentrations, impaired hematological parameters, and elevated inflammatory biomarkers compared with controls. ADMSC treatment reduced Anti-Tg Ab and TSH levels, restored FT3 and FT4 concentrations, improved hematological indices, decreased inflammatory markers, and increased immunoregulatory biomarkers toward control values.

Conclusions: ADMSCs attenuated autoimmune and inflammatory responses, improved thyroid function, and ameliorated hematological and immunological alterations in experimental HT. These findings support the potential of ADMSCs as a regenerative and immunomodulatory therapeutic strategy for Hashimoto's thyroiditis.

Keywords: Hashimoto Disease, Mesenchymal Stem Cells, Adipose Tissue, Autoimmune Thyroiditis, Wistar Rats

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Introduction

Hashimoto's thyroiditis (HT) is an autoimmune disorder characterized by chronic inflammation and progressive destruction of thyroid tissue. HT develops when the immune system loses tolerance to thyroid proteins, leading to lymphocytic infiltration. The resulting hypothyroidism causes fatigue, weight gain, and impaired physiological function.^{1,2} The pathophysiology of HT involves interactions between innate and adaptive immune responses. T cells infiltrate thyroid tissue and stimulate the release of IL-6, TNF- α , interferon- γ , and IL-17.³ These cytokines promote chronic inflammation, thyroid epithelial cell apoptosis, and thyroid fibrosis. In addition, B lymphocytes contribute to the production of autoantibodies against thyroid antigens, which is associated with reduced thyroid hormone levels.⁴

Hematological abnormalities associated with HT include alterations in red blood cell (RBC) and white blood cell

(WBC) parameters. Hypothyroidism reduces erythropoietin production and impairs erythropoiesis.^{5,6} Elevated levels of pro-inflammatory cytokines, increased autoantibody production, and abnormal T helper cell responses are also observed in HT.⁷

In recent years, mesenchymal stem cells (MSCs) have attracted considerable attention because of their immunomodulatory, anti-inflammatory, and tissue-repair properties. Adipose tissue-derived mesenchymal stem cells (ADMSCs) are a promising therapeutic candidate owing to their abundance, ease of isolation, rapid proliferation, and multilineage differentiation potential.⁸ ADMSCs can suppress excessive immune responses by secreting anti-inflammatory cytokines and regulatory growth factors, inhibiting T lymphocyte activation, and reducing the production of inflammatory cytokines.⁹

ADMSCs have demonstrated therapeutic potential in several autoimmune diseases through immunomodulation. They can reduce the infiltration of inflammatory cells and restore immune tolerance by regulating regulatory T (Treg) cells and inhibiting autoreactive lymphocytes.¹⁰ Therefore, investigating the effects of ADMSCs on HT is important for understanding their potential therapeutic role in modulating immune responses, improving hematological parameters, and reducing thyroid tissue injury.^{11,12}

The present study aimed to evaluate the hematological alterations associated with experimentally induced HT in Wistar rats and to investigate the accompanying immunological changes, including inflammatory cytokines and immune responses. In addition, the study aimed to assess the therapeutic effects of ADMSCs on hematological parameters in diseased rats and to determine their immunomodulatory role in reducing autoimmune and inflammatory responses associated with HT.

Materials and Methods

Animals and Study Design

A total of 40 female Wistar rats (8–10 weeks old; 180–220 g) were used. Female rats were selected because HT is more prevalent in females, and autoimmune thyroiditis models are generally more stable in female rodents. The rats were randomly divided into four groups ($n = 10$ per group): G1 received subcutaneous porcine thyroglobulin for HT induction; G2 received subcutaneous porcine thyroglobulin for HT induction and was subsequently treated with ADMSCs; G3 received ADMSCs only; and G4 received normal saline. All animals completed the experimental protocol, and no deaths, exclusions, or missing data occurred. Therefore, all 40 animals ($n = 10$ per group) were included in the final statistical analysis.

HT Induction

HT was induced in G1 and G2 using the experimental autoimmune thyroiditis (EAT) model. Porcine thyroglobulin (pTg) was dissolved in PBS and emulsified with Complete Freund's Adjuvant (CFA). On day 0, 150 μ g pTg was injected subcutaneously. On days 7 and 14, pTg emulsified with Incomplete Freund's Adjuvant (IFA) (Sigma, USA) was injected subcutaneously at the same dose (150 μ g). The disease developed over a period of 4 weeks. HT was successfully induced in all experimental animals (G1 and G2), as confirmed by elevated anti-thyroglobulin antibody (anti-Tg Ab) levels before treatment. Successful induction of HT was assessed by elevated anti-Tg Ab, increased TSH, and decreased FT3 and FT4 levels compared with controls. All animals in G1 and G2 developed HT. No animals were excluded because of mortality, failure of disease induction, incomplete treatment, missing samples, or invalid laboratory measurements.

Disease Confirmation

HT induction was confirmed by detecting anti-thyroglobulin antibodies (TgAb) using ELISA. In addition, thyroid function tests, including FT3, FT4, and TSH, were performed.

ADMSC Preparation and Treatment

ADMSCs were obtained from Cell Biologics (USA) and characterized by the provider using flow cytometry as positive for CD90, CD73, and CD105 and negative for CD34 and CD45. Cryopreserved ADMSCs were thawed rapidly in a 37 °C water bath and transferred aseptically into Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), 1% penicillin–streptomycin, and 2 mM L-glutamine (Sigma, USA). Cells were centrifuged at 1500 rpm for 5 min to remove the cryoprotective medium and then suspended in fresh culture medium. Cells were cultured in T-75 flasks and incubated at 37 °C in a humidified atmosphere containing 5% CO₂. The culture medium was replaced every 2–3 days until the cells reached 80–90% confluency. Cells were detached using 0.25% trypsin–EDTA solution (Gibco, USA), and viability was assessed using the trypan blue (Sigma, USA) exclusion assay. After confirmation of experimental HT at the end of the four-week induction period, ADMSC treatment was initiated. Rats in the ADMSC-treated group received an intravenous injection via the tail vein containing approximately 1×10^6 viable ADMSCs suspended in 0.5 ml sterile PBS. The dose was repeated 7 days after the first injection.¹¹

Hematological and Immunological Parameters

Hematological measurements using an automated hematology analyzer included RBC and WBC counts, Hb, PCV, and platelet count. ELISA was performed to measure pro-inflammatory cytokines (TNF- α , IL-6, IFN- γ , and IL-17) and anti-inflammatory cytokines (IL-10 and TGF- β). These biomarkers were selected because ADMSCs act mainly through immunomodulation.

Thyroid Hormone Evaluation

Serum FT3, FT4, and TSH concentrations were measured to assess thyroid function and therapeutic response.

Blood Sample Collection

At the end of the experimental period, blood samples were collected from anesthetized rats via cardiac puncture. Blood was divided into two portions. One portion was collected in EDTA tubes for hematological analysis. The second portion was collected in plain tubes for serum separation and immunological assays. Serum samples were centrifuged at 3000 rpm for 15 min and stored at –20 °C until analysis. Hematological parameters were evaluated using an

automated hematology analyzer. The following parameters were measured: RBC count, Hb, PCV/HCT, total WBC, platelet count, MCV, MCH, MCHC, RDW, and NLR. Blood smears stained with Wright–Giemsa (Sigma, USA) stain were examined microscopically for morphological evaluation of blood cells. TSH, FT4, FT3, and anti-Tg Ab were measured. The immunological parameters included TNF- α , IL-6, IL-17, IFN- γ , IL-10, C-reactive protein (CRP), and Erythrocyte sedimentation rate (ESR), which were additionally assessed as indicators of systemic inflammation.

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using SPSS software version 26. Differences among groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. A p value < 0.05 was considered statistically significant.

Results

For clarity, G1 represents the HT group, G2 the HT + ADMSC group, G3 the ADMSC-only group, and G4 the

healthy control group.

Thyroid Autoantibody and Hormone Levels

G1 showed significantly elevated anti-thyroglobulin antibody (anti-Tg Ab) levels (694 ± 12 ng/ml) compared with the control group (G4; 34 ± 2.1 ng/ml). In contrast, G2 and G3 exhibited markedly lower anti-Tg Ab levels (42.5 ± 1.8 ng/ml and 36 ± 1.9 ng/ml, respectively), which were not significantly different from the control. TSH levels were significantly higher in G1 (7.1 ± 0.1 pg/ml) than in the control (1.4 ± 0.6 pg/ml). TSH levels in G2 (2.2 ± 0.1 pg/ml) and G3 (1.3 ± 0.6 pg/ml) were significantly lower than in G1 and comparable to the control.

G1 exhibited the lowest FT3 concentration (1.2 ± 0.1 pg/ml), whereas G2 showed a significant increase (3.6 ± 0.1 pg/ml) compared with G1. G2 did not differ significantly from G4, while G3 and G4 had FT3 values of 4 ± 2 pg/ml. G1 also had the lowest FT4 concentration (0.08 ± 0 ng/dl) compared with the control. FT4 levels in G2 (0.39 ± 0 ng/dl) and G3 (0.334 ± 0.1 ng/dl) did not differ significantly from the control (0.321 ± 0.1 ng/dl), as shown in Table 1.

Table 1. Serum levels of anti-thyroglobulin antibody (Anti-Tg Ab), thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine (FT4) in the study groups

Biomarkers for HT	G1	G2	G3	G4
Anti-Thyroglobulin Antibody (Anti-Tg Ab) (ng/ml)	694 ± 12^A	42.5 ± 1.8^B	36 ± 1.9^B	34 ± 2.1^B
Thyroid-stimulating hormone (TSH) (pg/ml)	7.1 ± 0.1^A	2.2 ± 0.1^B	1.3 ± 0.6^B	1.4 ± 0.6^B
Free Triiodothyronine (FT3) (pg/ml)	1.2 ± 0.1^B	3.6 ± 0.1^A	4 ± 2^A	4 ± 2^A
Free Thyroxine (FT4) (ng/dl)	0.08 ± 0^B	0.39 ± 0^A	0.334 ± 0.1^A	0.321 ± 0.1^A

Values are presented as mean $\pm$ SD. G1, Hashimoto's thyroiditis (HT); G2, HT treated with adipose tissue-derived mesenchymal stem cells (ADMSCs); G3, ADMSC only; G4, healthy control. Comparisons were made within rows; means sharing the same superscript letter are not significantly different, whereas different superscript letters indicate significant differences ($p < 0.05$).

Table 2. Hematological Parameters in the Study Groups

Hematological biomarkers	G1	G2	G3	G4
HCT / PCV (%)	32 ± 1.5^B	38 ± 1^A	42 ± 1^A	43 ± 1.2^A
Mean Corpuscular Volume (MCV) (fL)	54 ± 1^B	57 ± 0.9^A	59 ± 0.8^A	55 ± 0.9^A
Mean Corpuscular Hemoglobin (MCH) (pg)	13 ± 1.1^B	22 ± 1^A	23 ± 1^A	19 ± 1.1^A
Red Cell Distribution Width (RDW) (%)	26 ± 1.6^A	17 ± 1.1^B	18 ± 0.9^B	15 ± 0.8^B
Absolute Neutrophil Count (cells/ μ l)(ANC)	$4.5 \times 10^3 \pm 0.2^A$	$1.4 \times 10^3 \pm 0.2^B$	$1.4 \times 10^3 \pm 0.2^B$	$1.3 \times 10^3 \pm 0.2^B$
Erythrocyte Sedimentation Rate (ESR) (mm/hr)	6.4 ± 0.6^A	2.4 ± 0.1^B	2.0 ± 0.1^B	2.1 ± 0.1^B
Neutrophil/Lymphocyte Ratio (ratio) (NLR)	1.2 ± 0.1^A	0.33 ± 0.2^B	0.29 ± 0.2^B	0.32 ± 0.1^B
Absolute lymphocyte count ($\times 10^3$ / μ l)(ALC)	$4.09 \times 10/\mu$ l	$4.61 \times 10/\mu$ l	$4.6 \times 10/\mu$ l	$4.3 \times 10/\mu$ l
Hemoglobin (Hb) (g/dl)	10.5 ± 0.3^B	15.2 ± 0.1^A	15.1 ± 0.2^A	14.4 ± 0.1^A
Platelet Count (PLT) (platelets/ μ l)	$811 \pm 25 \times 10^C$	$833 \pm 19 \times 10^{AB}$	$823 \pm 12 \times 10^B$	$853 \pm 11 \times 10^{3A}$

Values are presented as mean $\pm$ SD. G1, Hashimoto's thyroiditis (HT); G2, HT treated with adipose tissue-derived mesenchymal stem cells (ADMSCs); G3, ADMSC only; G4, healthy control. Comparisons were made within rows; means sharing the same superscript letter are not significantly different, whereas different superscript letters indicate significant differences ($p < 0.05$).

Hematological Parameters

HCT/PCV was lowest in G1 ($32 \pm 1.5\%$) compared with the control, whereas G2 showed a higher level ($38 \pm 1\%$) that was closer to the control. HCT/PCV values in G3 and G4 were $42 \pm 1\%$ and $43 \pm 1.2\%$, respectively. MCV was 54 ± 1 fL in G1, 57 ± 0.9 fL in G2, 59 ± 0.8 fL in G3, and 55 ± 0.9 fL in G4. MCH was reduced in G1 (13 ± 1.1 pg) compared with the control, while it was higher in G2 (22 ± 1 pg), G3 (23 ± 1 pg), and G4 (19 ± 1.1 pg). RDW was $26 \pm 1.6\%$ in

G1, compared with $17 \pm 1.1\%$ in G2, $18 \pm 0.9\%$ in G3, and $15 \pm 0.8\%$ in G4. ANC was elevated in G1 ($4.5 \times 10^3 \pm 0.2$ cells/ μ l) compared with G2 ($1.4 \times 10^3 \pm 0.2$ cells/ μ l), G3 ($1.4 \times 10^3 \pm 0.2$ cells/ μ l), and G4 ($1.3 \times 10^3 \pm 0.2$ cells/ μ l). ESR was 6.4 ± 0.6 mm/hr in G1, whereas it was 2.4 ± 0.1 mm/hr in G2, 2.0 ± 0.1 mm/hr in G3, and 2.1 ± 0.1 mm/hr in G4. NLR was elevated in G1 (1.2 ± 0.1) and lower in G2 (0.33 ± 0.2), G3 (0.29 ± 0.2), and G4 (0.32 ± 0.1). ALC was $4.09 \times 10^3/\mu$ l in G1, $4.61 \times 10^3/\mu$ l in G2, $4.6 \times 10^3/\mu$ l in G3,

and $4.3 \times 10^3/\mu\text{l}$ in G4. Hb increased in G2 compared with the control, with values of 10.5 ± 0.3 g/dl in G1, 15.2 ± 0.1 g/dl in G2, 15.1 ± 0.2 g/dl in G3, and 14.4 ± 0.1 g/dl in G4. Platelet counts were $811 \pm 25 \times 10^3/\mu\text{l}$ in G1, $833 \pm 19 \times 10^3/\mu\text{l}$ in G2, $823 \pm 12 \times 10^3/\mu\text{l}$ in G3, and $853 \pm 11 \times 10^3/\mu\text{l}$ in G4, as shown in Table 2.

Immunological Biomarkers

TNF- α levels were highest in G1 (91 ± 5 pg/ml) compared with the control (26 ± 2 pg/ml) and decreased in G2 (37 ± 4 pg/ml) and G3 (33 ± 3 pg/ml). IFN- γ was 77 ± 4 pg/ml in G1, 31 ± 3 pg/ml in G2, 24 ± 2 pg/ml in G3, and 21 ± 2 pg/ml in G4. IL-10 decreased to 13 ± 1 pg/ml in G1 compared with the control, while it was 22 ± 2 pg/ml in G2, 39 ± 3 pg/ml in G3, and 43 ± 3 pg/ml in G4. IL-17 was elevated in G1 (70 ± 4 pg/ml) compared with the control,

decreased in G2 (28 ± 3 pg/ml), and was 20 ± 2 pg/ml in G3 and 19 ± 1 pg/ml in G4. IL-35 was low in G1 (8.7 ± 1 pg/ml) and higher in G2 (24.1 ± 2 pg/ml), G3 (33.4 ± 2 pg/ml), and G4 (33.3 ± 3 pg/ml). TGF- β was 1.7 ± 0.2 ng/ml in G1, 4.6 ± 0.3 ng/ml in G2, 5.3 ± 0.4 ng/ml in G3, and 6 ± 0.5 ng/ml in G4. CRP levels were high in G1 (9.6 ± 0.6 mg/L) and decreased in G2 (3.3 ± 0.4 mg/L), G3 (2.6 ± 0.2 mg/L), and G4 (2.1 ± 0.2 mg/L). NF- κ B was increased in G1 (7.6 ± 0.5 ng/ml) compared with the control, while it was 3.4 ± 0.3 ng/ml in G2, 2.8 ± 0.2 ng/ml in G3, and 2.4 ± 0.2 ng/ml in G4. FoxP3 decreased in G1 (1.1 ± 0.1 ng/ml) compared with the control, and was 3.9 ± 0.2 ng/ml in G2, 4.4 ± 0.3 ng/ml in G3, and 5.6 ± 0.4 ng/ml in G4. NLRP3 levels increased in G1 (7 ± 0.4 ng/ml) compared with the control, while they were 3.3 ± 0.3 ng/ml in G2, 2.4 ± 0.2 ng/ml in G3, and 1.9 ± 0.1 ng/ml in G4, as shown in Table 3.

Table 3. Immunological and Inflammatory Biomarkers in the Study Groups

Immunological biomarkers	G1	G2	G3	G4
Tumor Necrosis Factor-alpha (TNF- α) (pg/ml)	91 ± 5^A	37 ± 4^B	33 ± 3^B	26 ± 2^C
Interferon-gamma (pg/ml)	77 ± 4^A	31 ± 3^B	24 ± 2^C	21 ± 2^D
Interleukin-10 (pg/ml)	13 ± 1^C	22 ± 2^B	39 ± 3^A	43 ± 3^A
Interleukin-17 (pg/ml)	70 ± 4^A	28 ± 3^B	20 ± 2^C	19 ± 1^C
Interleukin-35 (pg/ml)	8.7 ± 1^C	24.1 ± 2^B	33.4 ± 2^A	33.3 ± 3^A
Transforming Growth Factor-beta (TGF- β) (ng/ml)	1.7 ± 0.2^B	4.6 ± 0.3^A	5.3 ± 0.4^A	6 ± 0.5^A
C-Reactive Protein (mg/L)	9.6 ± 0.6^A	3.3 ± 0.4^B	2.6 ± 0.2^B	2.1 ± 0.2^B
Nuclear Factor kappa-B (NF- κ B) (ng/ml)	7.6 ± 0.5^A	3.4 ± 0.3^B	2.8 ± 0.2^B	2.4 ± 0.2^B
FoxP3 (Regulatory T-cell Marker) (ng/ml)	1.1 ± 0.1^C	3.9 ± 0.2^B	4.4 ± 0.3^B	5.6 ± 0.4^A
NLRP3 (ng/ml)	7 ± 0.4^A	3.3 ± 0.3^B	2.4 ± 0.2^C	1.9 ± 0.1^C

Values are presented as mean $\pm$ SD. G1, Hashimoto's thyroiditis (HT); G2, HT treated with adipose tissue-derived mesenchymal stem cells (ADMSCs); G3, ADMSC only; G4, healthy control. Comparisons were made within rows; means sharing the same superscript letter are not significantly different, whereas different superscript letters indicate significant differences ($p < 0.05$).

Discussion

The present study demonstrated that experimental Hashimoto's thyroiditis (HT) in Wistar rats was characterized by elevated anti-thyroglobulin antibody (anti-Tg Ab) and thyroid-stimulating hormone (TSH) levels, reduced free triiodothyronine (FT3) and free thyroxine (FT4) concentrations, hematological abnormalities, and an imbalance between pro-inflammatory and immunoregulatory mediators. ADMSC treatment attenuated these alterations, as evidenced by reduced anti-Tg Ab and TSH levels, improved thyroid hormone concentrations, partial restoration of hematological indices, decreased inflammatory biomarkers, and increased immunoregulatory mediators toward control values.

ADMSC administration can restore thyroid function by modulating the autoimmune response.¹¹ Our data are consistent with previous experimental studies reporting that mesenchymal stem cell (MSC) therapy improves thyroid parameters. MSC therapy decreases TgAb levels, restores T4 levels, and lowers TSH by suppressing inflammation.¹² ADMSCs have demonstrated immunomodulatory effects in autoimmune thyroid disease.¹³ MSC therapy restores endocrine

homeostasis in HT; however, apparent discrepancies among studies may be explained by several biological and methodological factors, including stem cell source, administered dose, transplantation route, disease severity, disease duration, and timing of outcome assessment, all of which can influence therapeutic efficacy.¹⁴ ADMSCs can stimulate paracrine immunomodulation and activate differentiation.¹⁵ They secrete anti-inflammatory cytokines that suppress autoreactive immune responses.¹⁶ Restoration of immune tolerance decreases thyroid antigen exposure, reduces autoantibody production, and gradually normalizes FT3, FT4, and TSH levels.¹⁷

The marked elevation of anti-Tg Ab and TSH levels in HT is accompanied by a decline in FT3 and FT4 levels.¹¹ Continuous exposure to thyroid antigens stimulates autoreactive B lymphocytes to produce pathogenic autoantibodies.¹⁸ As circulating thyroid hormone levels decline, the negative feedback mechanism on the pituitary gland is impaired, leading to compensatory hypersecretion of TSH to maintain thyroid hormone production.¹⁹ ADMSC therapy reduces anti-Tg Ab and TSH levels while restoring FT3 and FT4

levels.¹¹ MSC therapy attenuates autoimmune thyroid injury and improves thyroid endocrine function by decreasing thyroid autoantibody production, reducing lymphocytic infiltration, and normalizing serum TSH, FT3, and FT4 levels.¹² The ADMSC secretome contains hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), transforming growth factor-beta (TGF- β), and interleukin-10 (IL-10).^{11,12} This discrepancy suggests that suppression of autoimmune activity alone may not be sufficient to achieve immediate endocrine recovery, particularly when irreversible follicular destruction has already occurred or when residual functional thyroid tissue is limited.²⁰ Differences among studies include experimental animal strains, methods used to induce HT, disease duration before treatment initiation, stem cell source, cell dose, transplantation route and period, which influence therapeutic outcomes.²⁰

According to our results, HCT/PCV, MCV, MCH, and Hb were lower in the HT group than in the stem cell-treated group and the control. In contrast, RDW, ESR, neutrophil count, NLR, TNF- α , IL-17, CRP, NF- κ B, and NLRP3 were elevated in the HT group and decreased in the stem cell-treated group compared with the control. IL-10, IL-35, TGF- β , and FoxP3 were reduced in the HT group and increased after ADMSC treatment. Regarding platelet count, the HT group had a lower count than the control group, while G2 showed slightly higher counts.

The reductions in Hb, HCT/PCV, MCV, and MCH observed in the HT group were attributed to autoimmune thyroiditis. TNF- α suppresses erythropoietin production and inhibits erythroid progenitor cell proliferation in the bone marrow, leading to anemia of disease. In addition, hypothyroidism associated with HT reduces metabolic activity and oxygen demand, leading to decreased erythropoietin synthesis and impaired red blood cell production.^{21,22} Elevated RDW can be observed in ineffective erythropoiesis and anisocytosis caused by inflammatory stress.²³ Several investigators have demonstrated lower hemoglobin, reduced hematocrit, decreased MCV, and increased RDW in HT patients, suggesting that thyroid autoimmunity contributes to the development of anemia.^{5,24}

The marked increase in ESR, neutrophil count, CRP, and NLR in HT reflects systemic inflammation. Neutrophils represent a primary component of innate immunity and are rapidly recruited to inflammatory sites through cytokine-mediated signaling. Elevated NLR has recently emerged as a reliable marker of systemic inflammation and autoimmune disease severity. The increase in these inflammatory indices likely reflects the continuous immune-mediated inflammation of HT.²⁵

Significant elevations of TNF- α and NLRP3 were detected in HT. TNF- α is a major pro-inflammatory cytokine that promotes leukocyte recruitment and contributes to

thyrocyte apoptosis. Activation of NLRP3 stimulates the maturation and release of inflammatory cytokines. Increased NLRP3 expression has been observed in autoimmune disorders such as HT.²⁶ The mediators IL-10, IL-35, TGF- β , and FoxP3 are anti-inflammatory and immunoregulatory, and their decreased levels are associated with reduced autoimmune inflammation. IL-10, IL-35, TGF- β , and FoxP3 inhibit the autoimmune process in HT.²⁷

The potent immunomodulatory properties of MSCs explain the therapeutic effects observed following ADMSC administration. MSCs are known to suppress excessive immune activation by secreting anti-inflammatory mediators. They inhibit the proliferation of activated T lymphocytes and reduce the production of inflammatory cytokines.²⁸ The reductions observed in TNF- α , NLRP3, CRP, ESR, neutrophil count, and NLR after ADMSC therapy reflect suppression of inflammatory signaling pathways and attenuation of autoimmune thyroid injury.²⁹ MSC transplantation reduces levels of inflammatory cytokines, restores immune homeostasis, and reduces autoimmune-mediated tissue destruction.³⁰

The reductions in Hb, PCV, and MCH observed in HT may be attributed to inflammation. Pro-inflammatory cytokines such as IL-6, TNF- α , and interferon- γ interfere with iron metabolism, suppress erythropoietin production, and inhibit erythroid progenitor cell proliferation, thereby contributing to the development of anemia. The elevation in RDW supports the existence of disturbed erythropoiesis, as evidenced by an increase in erythrocyte size. The reduction in MCV, together with increased RDW, suggests disturbed erythropoiesis, although the predominant picture is consistent with inflammation-mediated suppression of erythropoiesis rather than nutritional deficiency.^{21,31,32}

The increased neutrophil count, ESR, and NLR detected in HT provide evidence of systemic immune activation. Neutrophils are among the earliest responders to inflammatory stimuli and contribute to the production of inflammatory mediators. ESR reflects increased acute-phase protein synthesis. NLR has emerged as a sensitive indicator of systemic inflammation and increases in HT. Therefore, the elevation of these parameters reflects the persistent autoimmune inflammatory process.^{33,34}

The favorable effects of ADMSC treatment on hematological and inflammatory indices are consistent with its immunomodulatory properties. ADMSCs secrete anti-inflammatory cytokines and trophic factors, which can suppress excessive immune activation and reduce pro-inflammatory cytokine production. The improvement in hemoglobin, PCV, MCH, and RDW observed in the stem cell-treated animals indicated reduced inflammation.^{33,35,36} Furthermore, ADMSCs suppress autoimmune inflammation by inhibiting effector T-cell activation. MSC therapy reduces inflammatory cell recruitment and cytokine production that affect thyroid tissue and bone marrow activity.^{28,37}

Our results showed that TNF- α levels were highest in HT, and MSC therapy decreased TNF- α levels. IFN- γ was increased in HT and decreased after ADMSC treatment. IL-10 decreased in G1, while G2 showed increased levels. IL-17 levels were elevated in G1 but reduced in G2. IL-35 was decreased in G1 and increased in the stem cell-treated group. TGF- β levels were lower in HT than in control, while G2 levels were higher than G1 levels. CRP levels increased in G1, whereas MSC therapy reduced them toward control values. NF- κ B was increased in G1 and reduced in G2. FoxP3 was decreased in G1, while G2 showed a normal level. NLRP3 levels increased in G1, whereas G2 showed a significant reduction but remained higher than control; many studies support these findings.³⁸

In the HT group, the expression levels of the anti-inflammatory mediators IL-10, IL-35, and TGF- β , and the regulatory T-cell transcription factor FoxP3, were suppressed compared with the control. These molecules are key immunoregulatory mediators essential for maintaining peripheral immune tolerance by activating and stabilizing regulatory T cells. Their depletion impairs Treg-mediated immunosuppressive activity, leading to the breakdown of immune tolerance and the uncontrolled activation of autoreactive immune cells.³⁹ Treatment with adipose tissue-derived mesenchymal stem cells (ADMSCs) restored the expression levels of IL-10, IL-35, TGF- β , and FoxP3. This restoration indicates the recovery of regulatory T-cell function. The enhanced immunoregulatory activity of Tregs suppresses pathogenic inflammatory responses and limits autoreactive immune cell activation. It attenuates the autoimmune attack against the thyroid gland, thereby reducing thyroid inflammation and promoting immune homeostasis.⁴⁰

Finally, this study has several limitations. The therapeutic effects of ADMSCs were evaluated using hematological and immunological parameters, without histopathological changes. The study focused on selected systemic biomarkers and did not investigate molecular mechanisms such as signaling pathways or gene expression profiles. Finally, the findings were obtained from an experimental Wistar rat model; caution is exercised when extrapolating these results to human HT. In addition, there was no HT + PBS vehicle control group. The main aim of the study was to evaluate the therapeutic effects of ADMSCs in rats with HT; therefore, the experimental design focused on comparing the HT group with the ADMSC-treated group and appropriate non-diseased controls. The absence of an HT + PBS group is a limitation. Future studies incorporating histopathological evaluation and molecular analyses are warranted to provide a more comprehensive assessment of the therapeutic efficacy of ADMSCs.

Conclusion

The present study demonstrated that ADMSC therapy

effectively ameliorated experimental Hashimoto's thyroiditis in rats, as evidenced by reduced anti-thyroglobulin antibody levels, improved thyroid hormone profiles, and ameliorated hematological parameters and immunoregulatory biomarkers. ADMSC administration attenuated autoimmune and inflammatory responses, resulting in improved thyroid function and partial restoration of immune homeostasis. These findings suggest that ADMSCs represent a promising regenerative and immunomodulatory strategy for the treatment of autoimmune thyroid disease. Further studies are warranted to confirm their clinical potential.

Authors' Contributions

All authors contributed substantially to the conception and design of the study. GKE performed the experimental work, animal handling, and laboratory analyses. NMM performed data collection and manuscript preparation. GKE and NMM contributed to the study design and data interpretation. GKE performed the manuscript revisions. All authors reviewed and approved the final manuscript.

Ethical Approval

All experimental procedures involving animals were performed in accordance with institutional guidelines for the care and use of laboratory animals and complied with internationally recognized ethical standards for animal experimentation. The study protocol was reviewed and approved by the Ethics Committee of the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq (Approval No. 1073; Date: 9 March 2026).

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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