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Histopathological changes and endoplasmic reticulum stress in type 1 diabetic mouse lung tissue

Cambios histopatológicos y el estrés del retículo endoplásmico en el tejido pulmonar de ratones con diabetes tipo 1

***Seçil Nazife Parlak*[1]*, *Şahin Aslan*[2]**

[1]*Ağrı İbrahim Çeçen University, Faculty of Medicine, Department of Histology and Embryology. Agri, Türkiye.*
[2]*Kafkas University, Faculty of Veterinary Medicine, Department of Histology and Embryology. Kars, Türkiye.*
**Corresponding author: seeparlak@gmail.com*

ABSTRACT

The aim of this study was to investigate the impact of type 1 diabetes on lung tissue using histopathological and immunohistochemical techniques, with a particular focus on whether it induces endoplasmic reticulum stress in the lung. The experiment utilized 18 male Balb/c mice, two months old with an average weight of 40 grams, randomly assigned to three groups: Control (n = 6), Sham (n = 6), and Streptozotocin diabetes group (n = 6). Diabetes was induced in the Streptozotocin group via a single intraperitoneal injection of 100 mg·kg^{-1} Streptozotocin. Following the experimental period, histopathological, immunohistochemical, and semi–quantitative analyses were conducted. Histopathological analysis revealed significant degeneration, apoptotic changes in epithelial cells and connective tissue, capillary dilation and inflammatory cell infiltration in the Streptozotocin group compared with the control and sham groups. Immunohistochemical analyses revealed a strong positive immunoreactivity for glucose regulated protein 78, nuclear factor kappa B and caspase–3 in type I and II pneumocytes, club cells, endothelial cells and alveolar macrophages in the Streptozotocin group. These observations imply the possible induction of endoplasmic reticulum stress in the lungs by type 1 diabetes and finally culminate into various histopathological alterations.

Key words: Diabetes mellitus; lung; endoplasmic reticulum stress; apoptosis; immunohistochemical markers

RESUMEN

El objetivo de este estudio fue investigar el impacto de la diabetes tipo 1 en el tejido pulmonar mediante técnicas histopatológicas e inmunohistoquímicas, con especial atención a si induce estrés del retículo endoplásmico en los pulmones. El experimento se realizó en 18 ratones machos Balb/c de dos meses de edad y un peso promedio de 40 gramos, asignados aleatoriamente a tres grupos: Control (n = 6), Sham (n = 6) y grupo diabético con estreptozotocina (n = 6). La diabetes fue inducida en el grupo estreptozotocina mediante una única inyección intraperitoneal de 100 mg·kg^{-1} de estreptozotocina. Al finalizar el período experimental, se realizaron análisis histopatológicos, inmunohistoquímicos y semicuantitativos. El análisis histopatológico reveló degeneración significativa, cambios apoptóticos en células epiteliales y tejido conectivo, dilatación capilar e infiltración de células inflamatorias en el grupo estreptozotocina en comparación con los grupos control y sham. El análisis inmunohistoquímico mostró una fuerte inmunorreactividad positiva para la proteína regulada por glucosa 78, factor nuclear kappa B y caspasa–3 en neumocitos tipo I y II, células club, células endoteliales y macrófagos alveolares del grupo estreptozotocina. Estas observaciones sugieren la posible inducción de estrés del retículo endoplásmico en los pulmones debido a la diabetes tipo 1, lo que finalmente conduce a diversas alteraciones histopatológicas.

Palabras clave: Diabetes mellitus; pulmón; estrés del retículo endoplasmático; apoptosis; marcadores inmunohistoquímicos

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INTRODUCTION

Diabetes mellitus refers to a chronic metabolic disorder that is associated with persistent hyperglycemia and marked systemic effects. The autoimmune–mediated destruction of pancreatic beta cells gives rise to the type 1 diabetes subtype and results in reduced or inhibited insulin secretion [1]. The disorder not only affects glucose metabolism but acts further by causing histopathological changes in various tissues, partly because of endoplasmic reticulum (ER) dysfunction [2].

The ER is one of the most critical organelles for cellular homeostasis. In addition, it is responsible for protein and lipid synthesis, calcium storage, detoxification, and its centralized role in carbohydrate and fat metabolism [3]. ER dysfunction could result in the accumulation of unfolded or misfolded proteins in its lumen, which in turn generates the condition termed ER stress. To cope with ER stress, cells activate the unfolded protein response (UPR), an adaptive process in response to ER imbalance [4]. The UPR usually halts protein synthesis, amplifies protein–folding capacity, and degrades misfolded proteins through specific signaling pathways like glucose regulated protein 78 (GRP–78), protein kinasa R–like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6), and inositol–requering enzyme 1 (IRE1) [5].

The regulator of the unfolder protein response (UPR) is GRP–78 which performs protein folding, calcium binding and degradation of misfolded proteins [6]. Activation of UPR involves dissociation of GRP–78 from ER stress sensors like PERK, ATF6 or IRE1. Specifically PERK phosphorylates the eukaryotic translation initiation factor 2A (eIF2α), which results in the activation of NF–κB, a common transcription factor engaged in pro–inflammatory and anti–apoptotic responses [7]. ER stress is also initiatory for the apoptosis pathways activated by caspase-3, which is often regarded as a terminal, common effector in pro–apoptotic pathways [8].

Among the many organs affected by type 1 diabetes, the kidney, the heart, and the brain are reported to be damaged by ER stress [9, 10, 11]. Additionally, the lung has perhaps been the organ least studied with respect to diabetes, but it is becoming evident that this organ could also be a target for some future diabetes–related complications [12].

Through evidence, diabetes can cause changes in the structure and function of lung tissues by inflammation, fibrosis, and the elevation in apoptosis under the influence of ER stress [9, 13]. Various types of reviews in the literature have shown histopathologic changes in the lung tissue along with type I diabetes [14, 15].

Therefore, the present descriptive study intended to investigate the histopathological and immunohistochemical effects of type 1 diabetes on lung tissue, particularly looking into the role of ER stress. Through the dissection of key markers GRP–78, NF–κB, and caspase-3 linked to this pathological mechanism, the present study aims to elucidate the underlying mechanisms behind the relationship between diabetes and pulmonary complications.

MATERIAL AND METHODS

This study was conducted in accordance with national guidelines for the care and use of laboratory animals. The experimental protocol was approved by the local ethics committee for experimental animals from Kafkas University on 03/09/2017 with the ethical committee number 2017–082.

Eighteen male Balb/c mice (*Mus musculus*) aged 2 months, weighing between 38–42 g, were obtained from Atatürk University Medical Experimental Application and Research Center. The mice were divided into three groups (n = 6 each): control, sham, and streptozotocin (STZ) diabetes groups. They were housed under controlled conditions (22 ± 2°C, 60–65% humidity, 12:12 h light/dark cycle) with *ad libitum* access to food and water.

Experimental procedure

Type 1 diabetes was induced in mice using STZ, a compound that selectively destroys pancreatic β–cells. A citrate buffer (pH 4.5) was prepared by dissolving 0.294 g of sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$, 294.1 g·mol^{-1}) in deionized water to a final volume of 100 mL. STZ (Sigma S0130–1G) was freshly dissolved in this buffer at a concentration of 40 mg·mL^{-1}.

A single intraperitoneal (i.p.) dose of 100 mg·kg^{-1} STZ was administered using a 1 mL insulin syringe, calculated based on individual body weight. The sham group received only 1 mL of citrate buffer (i.p.), while the control group received no treatment. Four hours after injection, all mice were provided with standard feed and drinking water.

Blood glucose levels were measured using a glucometer (Accu–Chek Active®, Roche Diagnostics GmbH, Germany) and test strips from tail vein samples. Baseline glucose was measured 1 h before STZ administration, and follow–up measurements were taken at 24, 48, and 72 h, and at 14 days (d) post–injection. Mice with blood glucose levels exceeding 220 mg·dL^{-1} were considered diabetic and included in the study [16, 17] (TABLE I).

After 30 d, diabetic mice were anesthetized using Xylazine (10 mg·kg^{-1}, i.p.) and Ketamine (60 mg·kg^{-1}, i.p.) and euthanized by cervical dislocation. Lung tissues were collected and fixed in 10% buffered formalin for histopathological and immunohistochemical evaluations.

TABLE I
Blood Glucose Levels for All Groups

Groups	Blood glucose levels (mg·dL^{-1})	F
Control (n: 6)	79 ± 4^{a}	
Sham (n: 6)	77 ± 7^{a}	99.305
STZ diabetes (n: 6)	309 ± 53^{b}	

STZ: Type 1 diabetes was induced in mice using streptozotocin. Data are expressed as mean ± standard deviation (SD), n: number of animals per group, mg·dL^{-1}: milligrams per deciliter, $^{a, b}$: different superscript letters indicate statistically significant differences between groups (P<0.05)

Histopathological examinations

Lung tissues were processed and embedded in paraffin following fixation in 10% buffered formalin. Sections with a thickness of 4 µm were obtained using a rotary microtome (Leica RM2235, Leica Biosystems, Germany), and stained with hematoxylin–eosin (H&E) [18] and Crossman's triple stain [19] or histopathological evaluation. Image analysis was performed using a light microscope (Olympus BX51, Olympus Corporation, Japan).

Immunohistochemical studies

The avidin–biotin–peroxidase complex method [20] was used to detect GRP–78 (Abcam, ab108615), NF–κB (Santa Cruz, sc–8008), and caspase–3 (Santa Cruz, sc–56053) proteins in lung tissue. Sections were treated with primary antibodies (anti–GRP78, 1:150; NF–κB p65, 1:100; caspase–3, 1:100) for 1 h at room temperature. After incubation with secondary antibodies, staining was visualized using diaminobenzidine (DAB (Thermo T A-125-HD)) [21]. Positive controls (liver tissue) and negative controls (lung tissue without primary antibody) were used to validate the method. Immunostained sections were scored semi–quantitatively on a scale of - to ++++ based on staining intensity [22].

Statistical analysis

Statistical analyses were performed using SPSS 18.0. (IBM, Chicago, IL, USA, SPSS 18 for Windows) Group comparisons were made using the Kruskal–Wallis test, followed by the Mann–Whitney U test with Bonferroni correction. Results were considered significant at $P<0.05$. The non–parametric tests (Kruskal–Wallis and Mann–Whitney U) were applied to the numerical equivalents (0–4) of the semi–quantitative immunoreactivity scores (ranging from – to ++++) to allow for statistical comparison [23].

RESULTS AND DISCUSSION

Lung histology appeared normal in the control group, and the sham group showed similar findings. In contrast, the STZ–induced diabetic group exhibited significant damage, including bronchial epithelial injury and mild submucosal degeneration. The interalveolar septa showed thickening, hyalinization, and collagen buildup, indicating fibrosis. Apoptotic features such as nuclear condensation in type I and II pneumocytes, cell shrinkage, and apoptotic–like bodies were evident in the alveolar lining.

Degeneration also affected the endothelial and smooth muscle layers of pulmonary vessels, with collagen deposits surrounding vascular and alveolar areas. Dilated capillaries with red blood cell infiltration and inflammatory cell presence, neutrophils, macrophages, lymphocytes, monocytes, and occasional multinucleated giant cells, were observed (FIGS. 1 and 2).

The histopathological outcomes align with previous findings indicating that diabetes contributes to lung inflammation and fibrotic changes, potentially mediated by oxidative stress stemming from chronic hyperglycemia [24]. The interalveolar septal thickening and hyaline material suggest extracellular matrix remodeling, a characteristic process seen in diabetic pulmonary complications [25]. Moreover, the infiltration of immune cells, particularly neutrophils, macrophages, and lymphocytes, reflects

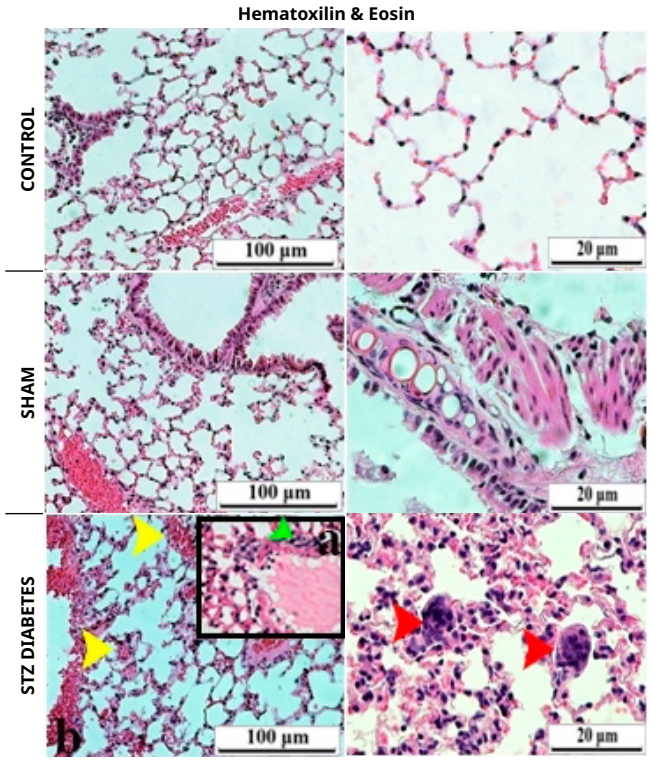

FIGURE 1. Hematoxylin and eosin (H&E) staining of lung tissues from all groups (100× and 400×). Yellow arrowhead: capillary dilation; green arrowhead: migration of neutrophils and lymphocytes from the vessel wall; red arrowhead: multinucleated giant cell. Scale bars = 100 µm and 20 µm

an active inflammatory response, further supporting the concept of "diabetic lung" pathology as previously described in the literature [26].

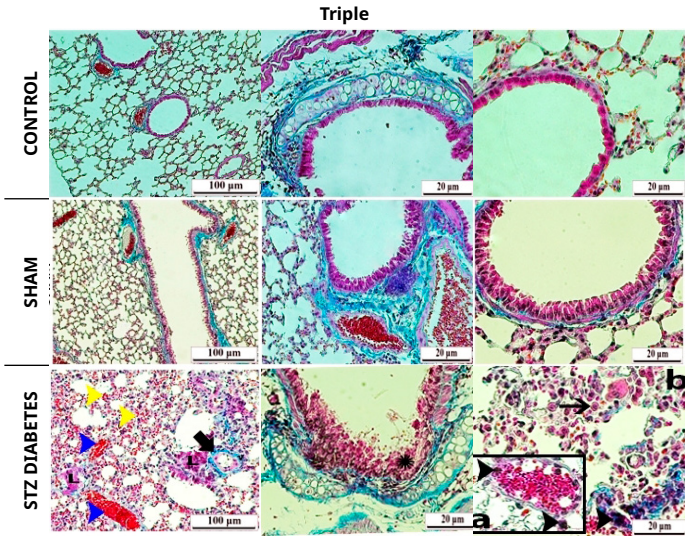

FIGURE 2. Triple staining of lung tissues from all groups (100× and 400×). ∗: Degenerative lamina epithelialis, L: increased alveolar septal thickness, black arrow: alveolar macrophage, black arrowhead: macrophage, bold black arrow: collagen deposition, blue arrowhead: congestion, yellow arrowhead: erythrocyte infiltration. Scale bars = 100 µm and 20 µm

In the control group, GRP–78 immunoreactivity was minimal and limited to alveolar macrophages. The sham group showed a similar pattern, with moderate staining in these cells. In the STZ–induced diabetes group, GRP–78 expression increased notably moderate in endothelial cells, strong in club cells and type I pneumocytes, and very strong in type II pneumocytes and alveolar macrophages.

Positive controls confirmed staining in hepatocytes, while no signal was detected in the negative controls. Semi–quantitative analysis showed no significant differences between control and sham groups in type II pneumocytes, club cells, or endothelial cells ($P>0.05$), but significant increases were found in type I pneumocytes and alveolar macrophages ($P<0.05$). All cell types showed significantly higher GRP–78 levels in the diabetic group compared to control and sham groups ($P<0.05$). (FIGS. 3 and 4, TABLE II).

The GRP–78, a key marker of ER stress, showed increased immunoreactivity in type I and II pneumocytes, club cells, endothelial cells, and alveolar macrophages in the STZ–induced diabetes group. This study provides direct immunohistochemical evidence of ER stress activation in diabetic lung tissue, supporting the idea that ER stress contributes to diabetes–related pulmonary changes. The upregulation of GRP–78 likely represents a compensatory response to hyperglycemia–induced protein misfolding and ER dysfunction [27]. However, prolonged ER stress is known to promote apoptosis and fibrosis, both of which were observed in the diabetic group's histopathological findings [28, 29].

In the control group, NF–κB immunoreactivity was minimal across all pulmonary cell types, while the sham group showed moderate immunoreactivity. In the STZ–induced diabetes group, NF–κB expression was markedly elevated—intense in endothelial cells and very intense in club cells, type I and II pneumocytes, and alveolar macrophages.

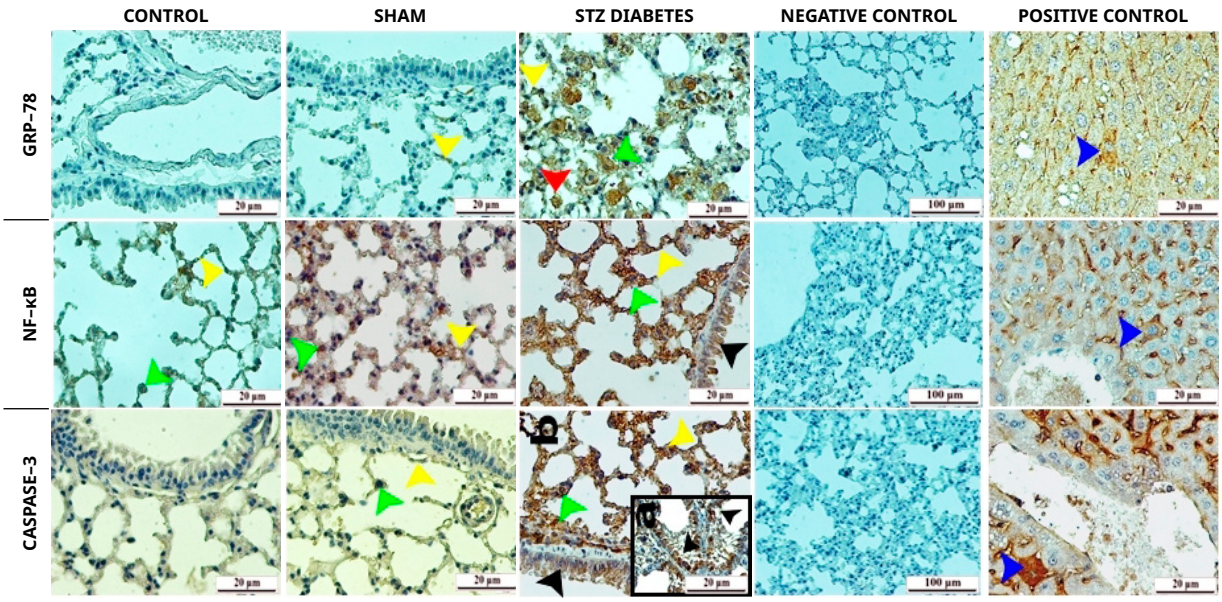

FIGURE 3. Immunohistochemical localization of GRP–78, NF–κB, and Caspase–3 in all groups, including negative and positive (liver tissue) controls (100× and 400×). Yellow arrowhead: positivity in type I pneumocytes, green arrowhead: positivity in type II pneumocytes, black arrowhead: positivity in club cells, red arrowhead: positivity in alveolar macrophages, blue arrowhead: positivity in hepatocytes. Scale bars = 100 μm and 20 μm

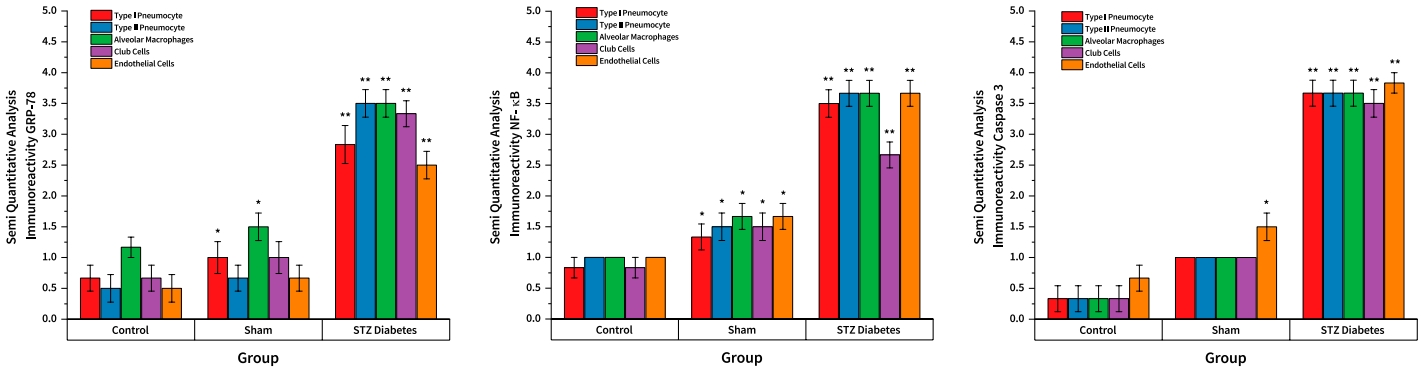

FIGURE 4. Statistical analysis of the semiquantitative assessments of GRP–78, NF–kB and Caspase 3 immune reactivity in lung tissues for all groups

TABLE II
Results of semiquantitative analysis of GRP–78 immunoreactivity between groups

Groups of GRP78 IR density	Control	Sham	STZ Diabetes
Club cells	–	–	+++
Endothelial cells	–	–	++
Type 1 pneumocyte	–	+	+++
Type 2 pneumocyte	–	–	++++
Alveolar macrophage	+	++	++++

STZ: Type 1 diabetes was induced in mice using streptozotocin. Data represent the semiquantitative immunoreactivity (IR) scores of GRP–78 expression in various lung cell types, - : no immunoreactivity, +: mild, ++: moderate, +++: strong, ++++: very strong immunoreactivity, IR: immunoreactivity, GRP–78: Glucose–regulated protein 78, n = 6 animals per group

Positive controls confirmed NF–κB staining in hepatocytes, whereas no staining was observed in negative controls. Statistical analysis showed significant differences in NF–κB immunoreactivity among all groups (P<0.05), with the STZ group exhibiting significantly higher expression levels compared to both the control and sham groups (FIGS. 3 and 4, TABLE III).

TABLE III
Results of semiquantitative analysis of NF–kB immunoreactivity between groups

Groups of NF–kB IR density	Control	Sham	STZ Diabetes
Club cells	+	++	++++
Endothelial cells	+	++	+++
Type 1 pneumocyte	+	++	++++
Type 2 pneumocyte	+	++	++++
Alveolar macrophage	+	++	++++

STZ: Type 1 diabetes was induced in mice using streptozotocin. Data represent the semiquantitative immunoreactivity (IR) scores of NF–kB expression in various lung cell types, - : no immunoreactivity, +: mild, ++: moderate, +++: strong, ++++: very strong immunoreactivity, IR: immunoreactivity, NF–kB: Glucose–regulated protein 78, n = 6 animals per group

The marked increase in NF–κB expression observed in the STZ–induced diabetes group indicates that chronic inflammation is a key contributor to diabetes–related pulmonary injury. NF–κB is a pivotal transcription factor that regulates the expression of numerous pro–inflammatory cytokines and has been implicated in the pathogenesis of various diabetic complications [30].

Consistent with previous studies, the present findings support the notion that hyperglycemia–induced NF–κB activation promotes the recruitment and accumulation of inflammatory cells in lung tissue, thereby aggravating structural damage [31]. Moreover, NF–κB activation is closely associated with endoplasmic reticulum stress, a mechanism reflected in the concurrent upregulation of GRP–78 in diabetic lung tissue [32].

Beyond its role in inflammation, NF–κB also modulates apoptotic pathways by regulating the expression of both pro–apoptotic and anti–apoptotic proteins, linking chronic inflammation to tissue degeneration in the diabetic lung [33].

In the control group, caspase–3 expression was absent, whereas the sham group exhibited minimal immunoreactivity. In contrast, the STZ–induced diabetes group showed a marked increase, with very intense caspase–3 immunoreactivity in pulmonary tissues. Positive control sections demonstrated distinct caspase–3–positive hepatocytes, while no staining was observed in the negative controls, confirming the specificity of the immunohistochemical procedure. Semi–quantitative evaluation revealed statistically significant differences among all groups (P<0.05). Caspase–3 immunoreactivity scores were significantly higher in the STZ diabetes group compared with both the control and sham groups (FIGS. 3 and 4, TABLE IV).

TABLE IV
Results of semiquantitative analysis of Caspase–3 immunoreactivity between groups

Groups of NF–kB IR density	Control	Sham	STZ Diabetes
Club cells	-	+	++++
Endothelial cells	-	+	++++
Type 1 pneumocyte	-	+	++++
Type 2 pneumocyte	-	+	++++
Alveolar macrophage	-	+	++++

STZ: Type 1 diabetes was induced in mice using streptozotocin. Data represent the semiquantitative immunoreactivity (IR) scores of NF–kB expression in various lung cell types, - : no immunoreactivity, +: mild, ++: moderate, +++: strong, ++++: very strong immunoreactivity, IR: immunoreactivity, NF–kB: Glucose–regulated protein 78, n = 6 animals per group

Caspase–3 which acts as an apoptotic key–effector showed high values in STZ diabetic cohort indicating increased cell death. Presence of features like nuclear condensation and cell shrinkage in alveolar epithelial cells strongly substantiate this hypothesis. Earlier, increased apoptosis in the lung tissues with alterations in structural–functional identity due to diabetes–induced oxidative stress, were proven [34].

NF–κB the present activation has also been shown to control apoptosis, which indicates a relationship between an inflammation and an apoptotic pathway [35]. This amplifies the heavy orchestration, wherein ER stress would activate NF–kappa B which aggravates inflammation and already activates apoptosis via caspase–3, which in the eventuality leads to degeneration of the lung tissue.

It is important to note that the present study has a descriptive and correlational design. Accordingly, the associations identified between type 1 diabetes, ER stress, and pulmonary histopathological alterations do not establish a direct causal relationship. As a projection for future research, the use of specific ER stress inhibitors would be valuable to determine whether pharmacological modulation of this pathway can causally influence the inflammatory and apoptotic processes observed in diabetic lung tissue.

Like all experimental studies, the present research has inherent limitations. The findings are derived from a controlled animal model and may not fully represent the complexity of human physiology. Moreover, the molecular assessments were restricted to selected pathways related to ER stress, inflammation, and apoptosis, which may not capture the full spectrum of mechanisms involved.

Additionally, while the present study employed a descriptive and semi–quantitative approach, future research could benefit from incorporating quantitative digital image analysis techniques—such as morphometric measurements, pixel intensity quantification, or software–based evaluation using tools like ImageJ or QuPath—to obtain more objective and reproducible data regarding histopathological and immunohistochemical alterations in diabetic lung tissue. Therefore, further comprehensive and translational investigations are required to expand and validate these observations.

CONCLUSION

This study provides clear evidence that type 1 diabetes induces significant structural and molecular alterations in lung tissue, primarily through the activation of ER stress. The upregulation of GRP–78 confirms engagement of the unfolded protein response, while increased NF–κB and caspase-3 expressions suggest that ER stress contributes to both inflammatory and apoptotic processes. These findings support the presence of a pathological crosstalk between ER stress and inflammation, which may play a role in diabetes–related pulmonary damage.

Conflict of interest statement

The authors declare that there is no conflict of interest.

BIBLIOGRAPHIC REFERENCES

[1] Leslie KA, Lekka C, Richardson SJ, Russell MA, Morgan NG. Regulation of STAT1 signaling in human pancreatic β–Cells by the Lysine Deacetylase HDAC6: A new therapeutic opportunity in type 1 Diabetes? Diabetes [Internet]. 2024; 73(9):1473–1485. doi: https://doi.org/qkpk

[2] Abdel–Ghaffar A, Elhossary GG, Mahmoud AM, Elshazly AHM, Hassanin OA, Saleh A, Mansour SM, Metwally FG, Hanafy LK, Karam SH, Darweesh N, Ata AM. Effects of 4–phenylbutyric acid on the development of diabetic retinopathy in diabetic rats: regulation of endoplasmic reticulum stress–oxidative activation. Arch. Physiol. Biochem. [Internet]. 2023; 129(4):964–974. doi: https://doi.org/qkpm

[3] Chen S, Novick P, Ferro–Novick S. ER structure and function. Curr. Opin. Cell Biol. [Internet]. 2013; 25(4):428–433. doi: https://doi.org/gjfw25

[4] Almanza A, Carlesso A, Chintha C, Creedican S, Doultsinos D, Leuzzi B, Luís A, McCarthy N, Montibeller L, More S, Papaioannou A, Püschel F, Sassano ML, Skoko J, Agostinis P, de Belleroche J, Eriksson LA, Fulda S, Gorman AM, Healy S, Kozlov A, Muñoz–Pinedo C, Rehm M, Chevet E, Samali A. Endoplasmic reticulum stress signalling – from basic mechanisms to clinical applications. FEBS J. [Internet]. 2019; 286(2):241–278. doi: https://doi.org/gf4ch9

[5] Malhotra JD, Kaufman RJ. The endoplasmic reticulum and the unfolded protein response. Semin. Cell Dev. Biol. [Internet]. 2007; 18(6):716–731. doi: https://doi.org/c3bstq

[6] Pfaffenbach KT, Lee AS. The critical role of GRP78 in physiologic and pathologic stress. Curr. Opin. Cell. Biol. [Internet]. 2011; 23(2):150–156. doi: https://doi.org/djz3pr

[7] Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM. Suppression of TNF–alpha–induced apoptosis by NF–kappaB. Science [Internet]. 1996; 274(5288):787–789. doi: https://doi.org/b5xc4s

[8] Hitomi J, Katayama T, Taniguchi M, Honda A, Imaizumi K, Tohyama M. Apoptosis induced by endoplasmic reticulum stress depends on activation of caspase–3 via caspase–12. Neurosci. Lett. [Internet]. 2004; 357(2):127–130. doi: https://doi.org/dhfwt5

[9] Liu G, Sun Y, Li Z, Song T, Wang H, Zhang Y, Ge Z. Apoptosis induced by endoplasmic reticulum stress involved in diabetic kidney disease. Biochem. Biophys. Res. Commun. [Internet]. 2008; 370(4):651–656. doi: https://doi.org/fr53z8

[10] Zhao Y, Yan Y, Zhao Z, Li S, Yin J. The dynamic changes of endoplasmic reticulum stress pathway markers GRP78 and CHOP in the hippocampus of diabetic mice. Brain Res. Bull. [Internet]. 2015; 111:27–35. doi: https://doi.org/f626t2

[11] Galán M, Kassan M, Choi SK, Partyka M, Trebak M, Henrion D, Matrougui K. A novel role for epidermal growth factor receptor tyrosine kinase and its downstream endoplasmic reticulum stress in cardiac damage and microvascular dysfunction in type 1 diabetes mellitus. Hypertension [Internet]. 2012; 60(1):71–80. doi: https://doi.org/qkpn

[12] Teodoro JS, Nunes S, Rolo AP, Reis F, Palmeira CM. Therapeutic options targeting oxidative stress, mitochondrial dysfunction and inflammation to hinder the progression of vascular complications of Diabetes. Front. Physiol. [Internet]. 2019; 9:1857. doi: https://doi.org/qkpp

[13] Tanjore H, Blackwell TS, Lawson WE. Emerging evidence for endoplasmic reticulum stress in the pathogenesis of idiopathic pulmonary fibrosis. Am. J. Physiol. Lung Cell. Mol. Physiol. [Internet]. 2012; 302(8):L721–729. doi: https://doi.org/fxr7zc

[14] Kida K, Utsuyama M, Takizawa T, Thurlbeck WM. Changes in lung morphologic features and elasticity caused by streptozotocin–induced diabetes mellitus in growing rats. Am. Rev. Respir. Dis. [Internet]. 1983; 128(1):125–131. doi: https://doi.org/qkpq

[15] Popov D, Simionescu M. Alterations of lung structure in experimental diabetes, and diabetes associated with hyperlipidaemia in hamsters. Eur. Respir. J. [Internet]. 1997; 10(8):1850–1858. doi: https://doi.org/d27qg3

[16] Elderbi M, Omar A, Omar H, Elburi A, Ibrahim M. Consequences of alloxan–induced diabetes on hematological and hepatic parameters in albino mice. Khalij–Libya J. Dent. Med. Res. [Internet]. 2025; 9(1)38–43.doi: https://doi.org/qkpr

[17] Deprem T, Yıldız SE, Sari EK, Bingol SA, Tasci SK, Aslan S, Sozmen M, Nur G. Distribution of glutathione peroxidase 1 in liver tissues of healthy and diabetic rats treated with capsaisin. Biotech. Histochem. [Internet]. 2015; 90(1):1–7. doi: https://doi.org/qkps

[18] Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. CSH Protoc. [Internet]. 2008; 2008:pdb.prot4986. doi: https://doi.org/cq3zx8

[19] Bancroft J, Cook H. Manual of Histological Techniques. New York (USA): Churchill Livingstone; 1984.

[20] Hsu SM, Raine L, Fanger H. Use of avidin–biotin–peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. [Internet]. 1981; 29(4):577–580. doi: https://doi.org/b573dv

[21] Shu SY, Ju G, Fan LZ. The glucose oxidase–DAB–nickel method in peroxidase histochemistry of the nervous system. Neurosci. Lett. [Internet]. 1988; 85(2):169–171. doi: https://doi.org/bfvmmc

[22] Okihiro MS, Hinton DE. Partial hepatectomy and bile duct ligation in rainbow trout (*Oncorhynchus mykiss*): histologic, immunohistochemical and enzyme histochemical characterization of hepatic regeneration and biliary hyperplasia. Toxicol. Pathol. [Internet]. 2000; 28(2):342–356. doi: https://doi.org/bdh4pf

[23] Bencze J, Szarka M, Kóti B, Seo W, Hortobágyi TG, Bencs V, Módis LV, Hortobágyi T. Comparison of semi–quantitative scoring and artificial intelligence aided digital image analysis of chromogenic immunohistochemistry. Biomolecules [Internet]. 2021; 12(1):19. doi: https://doi.org/gpfs9p

[24] Wang D, Ma Y, Tong X, Zhang Y, Fan H. Diabetes mellitus contributes to idiopathic pulmonary fibrosis: A review from clinical appearance to possible pathogenesis. Front. Public. Health. [Internet]. 2020; 8:196. doi: https://doi.org/gp78v9

[25] Hu Y, Ma Z, Guo Z, Zhao F, Wang Y, Cai L, Yang J. Retraction Note to: Type 1 diabetes mellitus is an independent risk factor for pulmonary fibrosis. Cell Biochem. Biophys. [Internet]. 2023; 81(2):377. doi: https://doi.org/qkpt

[26] Tiengo A, Fadini GP, Avogaro A. The metabolic syndrome, diabetes and lung dysfunction. Diabetes Metab. [Internet]. 2008; 34(5):447–454. doi: https://doi.org/c6fp8w

[27] Lam TYW, Nguyen N, Peh HY, Shanmugasundaram M, Chandna R, Tee JH, Ong CB, Hossain MZ, Venugopal S, Zhang T, Xu S, Qiu T, Kong WT, Chakarov S, Srivastava S, Liao W, Kim JS, Teh M, Ginhoux F, Fred–Wong WS, Ge R. ISM1 protects lung homeostasis via cell–surface GRP78–mediated alveolar macrophage apoptosis. Proc. Natl. Acad. Sci. USA. [Internet]. 2022; 119(4):e2019161119. doi: https://doi.org/gqkmq6

[28] Kropski JA, Blackwell TS. Endoplasmic reticulum stress in the pathogenesis of fibrotic disease. J. Clin. Invest. [Internet]. 2018; 128(1):64–73. doi: https://doi.org/gcrx8s

[29] Chipurupalli S, Samavedam U, Robinson N. Crosstalk Between ER Stress, Autophagy and Inflammation. Front. Med. [Internet]. 2021; 8:758311. doi: https://doi.org/gpjnqx

[30] Patel S, Santani D. Role of NF–kappa B in the pathogenesis of diabetes and its associated complications. Pharmacol. Rep. [Internet]. 2009; 61(4):595–603. doi: https://doi.org/gh6vh4

[31] Raguraman R, Srivastava A, Munshi A, Ramesh R. Therapeutic approaches targeting molecular signaling pathways common to diabetes, lung diseases and cancer. Adv. Drug. Deliv. Rev. [Internet]. 2021; 178:113918. doi: https://doi.org/gprtxn

[32] Chaudhari N, Talwar P, Parimisetty A, Lefebvre–d'Hellencourt C, Ravanan P. A molecular web: endoplasmic reticulum stress, inflammation, and oxidative stress. Front. Cell. Neurosci. [Internet]. 2014; 8:213. doi: https://doi.org/gpvmbj

[33] Radhakrishnan SK, Kamalakaran S. Pro–apoptotic role of NF–kappaB: implications for cancer therapy. Biochim. Biophys. Acta (BBA). [Internet]. 2006; 1766(1):53–62. doi: https://doi.org/b2zjcv

[34] Zheng H, Wu J, Jin Z, Yan LJ. Potential biochemical mechanisms of lung injury in Diabetes. Aging Dis. [Internet]. 2017; 8(1):7–16. doi: https://doi.org/qkpv

[35] Kucharczak J, Simmons MJ, Fan Y, Gélinas C. To be, or not to be: NF–κB is the answer—role of Rel/NF–κB in the regulation of apoptosis. Oncogene [Internet]. 2003; 22(56):8961–8982. doi: https://doi.org/dq49ph