Relationship between oxidative stress in the rotator cuff and transcutaneous AGEs measurement in diabetic rats

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INTRODUCTION: The incidence of rotator cuff tears in the shoulder increase with age, causing pain at night, muscle weakness, and a limited range of motion, and are associated with decreased ADL and shoulder joint pain during daily activities. The prevalence of rotator cuff tears is also known to increase in diabetic patients. The mechanism of tendon degenerative change and the subsequent injury in diabetes is thought to be influenced by the deposition of advanced glycation end-products (AGEs) due to oxidative stress in tissues [1]. Therefore, monitoring AGEs in tissues could enable the assessment of the pathological status of the rotator cuff and the risk of tearing. Recent studies have shown that a non-invasive method of measuring AGEs, a transcutaneous fluorescence intensity measuring device, can be used to measure AGEs deposited in skin tissue, including the epithelium or dermis [2]. This non-invasive approach has the potential to predict the status of oxidative stress in individual organs. It has also been reported that the accumulation of AGEs in skin tissue is associated with reduced skeletal muscle mass and bone density and may serve as a biomarker for lower back and lower extremity pain and numbness [3]. The purpose of this study was to investigate the association between the degree of AGEs accumulation and its histological properties in the diabetic rotator cuff as well as the value of transcutaneous fluorescence intensity.

METHODS: Nine-week-old male Sprague Dawley rats were randomly divided into control (n = 10) and diabetic (n = 10) groups. At 19 weeks of age, the diabetic group received a single intraperitoneal injection of streptozotocin (STZ) (60 mg/kg) dissolved in citrate buffer (pH 4.0). The control group received the same volume of citrate buffer intraperitoneally. Approximately 8 and 16 weeks after STZ administration, five rats in each group were euthanized by the intraperitoneal administration of sodium pentobarbital. After demineralization and paraffin embedding, a 5-μm-thick thin-slice specimen of the anterior forehead, including the supraspinatus tendon attachment area, was prepared. For transcutaneous AGEs measurements, as in previous report [4], a device consisting of a light-emitting diode light source, a spectral device system with a 2048-pixel Charge Coupled Device linear image sensor and grating, and a two-way silica-based optical fiber was used for each rat auricular estimated by measuring autofluorescence from the skin. Nine measurements were taken at 8 and 16 weeks after STZ administration prior to euthanasia, and the average of the values was used. Sections were treated with 3% H2O2 for 10 min and normal horse serum for 30 min and incubated overnight at 4 °C in the presence of primary antibodies against AGEs and type I collagen. Normal IgG was used as a negative control. The samples were then incubated with secondary antibodies for 30 min, followed by incubation with 3,3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide for 10 min at room temperature. Finally, the sections were stained with hematoxylin for 1 min. The quantitative evaluation of AGEs accumulation in the rotator cuff attachment area was performed with sections stained for AGEs antigens using immunohistochemical staining and a microscope with a 10x objective lens. The region of interest (ROI) was the supraspinatus muscle tendon from the medial border to the attachment of the greater tubercle of the humeral head. In the ROI, stained areas were measured semi-automatically using WinROOF 2015 software. Statistical differences between groups were analyzed using the Mann-Whitney U test. The threshold of significance was set at $p < 0.05$.

RESULTS: Transcutaneous fluorescence intensity measurements using auricular skin revealed no significant difference between the control and diabetic groups before STZ administration (control:1.34 ± 0.17. diabetic: 1.33 ± 0.25, p = 0.65). At 8 weeks after STZ administration, there was no significant difference between the control group and the diabetic group (control:1.39 ± 0.18, diabetic:1.48 ± 0.10, P = 0.35). In contrast, the diabetic group exhibited a significant increase in fluorescence intensity compared with the control group after 16 weeks (control:1.43 ± 0.18, diabetic:2.14 ± 0.22, p = 0.01) **(Fig.1)**. All groups exhibited immunohistochemical expression of AGEs at the attachment site of the rotator cuff **(Fig.2)**. No statistically significant difference was observed in the area of AGEs staining in the diabetic group when compared to the control at 8 weeks after STZ administration, although there was a trend toward a higher percentage in the diabetic group (control: mean 28.3±3.4%, diabetes: mean 32.6±3.8%, p = 0.12). At 16 weeks after administration, the diabetic group exhibited significantly enhanced immunohistological expression of AGEs at the rotator cuff attachment site compared with the control group (control: mean 30.1 ± 5.6%, diabetes: mean 36.8 ± 4.0%, p = 0.03) **(Fig.3)**. No significant differences in the immunohistochemical expressions of collagen I and IgG were observed between the groups **(Fig.3)**.

DISCUSSION: In this study, we evaluated the transcutaneous fluorescence intensity in rat auricular tissue and the accumulation of AGEs in rotator cuff tissue using a rat diabetes mellitus model. Our data demonstrated increased AGEs deposition in the rotator cuff area due to prolonged hyperglycemia caused by diabetes mellitus, and a positive correlation between histological pathology and transcutaneous fluorescence intensity. This suggests that it is possible to infer the accumulation of AGEs in rotator cuff tissue non-invasively using transcutaneous fluorescence intensity. It may also be used to predict the risk of AGEs-induced degeneration of rotator cuff tissue due to chronic inflammation and development of non-traumatic rotator cuff injuries. Furthermore, for patients with suspected rotator cuff injuries or those undergoing surgery for rotator cuff tears, transcutaneous skin fluorescence intensity measurements in addition to magnetic resonance imaging and ultrasound, which are widely used today, could have the potential to provide information on tissue quality and assess the preoperative risk of possible postoperative re-tears after repair, as well as the risk of developing rotator cuff injuries.

SIGNIFICANCE/CLINICAL RELEVANCE: Transcutaneous skin fluorescence intensity measurements are useful as a non-invasive method for measuring oxidative stress at the rotator cuff, and could potentially be used to assess the risk of rotator cuff tissue degeneration, rupture, and re-tear after rotator cuff repair surgery.

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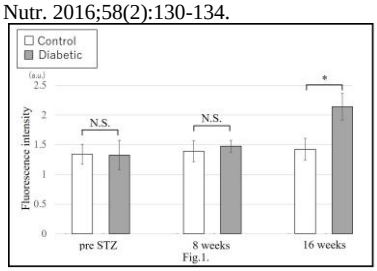

Fig.1.

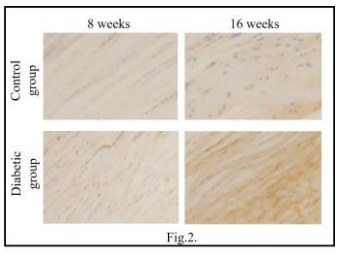

Fig.2.

STZ administration	After 8 weeks		After 16 weeks	
	Control (n=5)	Diabetic (n=5)	Control (n=5)	Diabetic (n=5)
AGEs (%)	28.3 ± 3.4	32.6 ± 3.8	30.1 ± 5.6	36.8 ± 4.0*
Collagen 1 (%)	68.9 ± 7.1	67.9 ± 9.7	66.3 ± 6.2	69.3 ± 8.2
Normal IgG (%)	7.6 ± 3.8	7.3 ± 3.3	7.1 ± 2.4	7.8 ± 3.3

Fig.3.

Fig.1. Changes in percutaneous fluorescence intensity of the auricle skin after STZ administration.
Fig.2. Immunohistochemical expression of advanced glycation end products (AGEs).
Fig.3. Quantitative immunohistochemistry in supraspinatus tendons after STZ administration.