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Tympanometry Comparison of Diabetic Type I and Diabetic Type II Rats

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Abstract

Hearing impairment affects over two-thirds of adults with diabetes. We investigated whether rat models of type 1 and type 11 diabetes display impaired auditory function. Tympanometry measurements were conducted in Sprague-Dawley rats (control, n = 20), streptozotocin-induced type I diabetic Sprague-Dawley rats (n = 20) at 42 - 56 days old; Zucker rats (Hos: ZFDM-Lean (fa/+, n = 20) and Zucker Type 2 Diabetic rats (ZFDM (Hos: ZFDM-fa/fa); n = 20)), 90 days old. All rats were male. Control animals had normal type A tympanograms. Tweny one (75%) of the tympanic membranes in the diabetic type I group produced abnormal tympanograms: 46% were type B, 28% had no peak found, and 1% were type C. The ear canal measurements were lower in the left ear in type I mice (0.19 ± 0.07) and higher in the left ear for type II mice (0.23 ± 0.15 ml) compared to the controls of 0.39 ± 0.14 ml) and (0.2 ± 0.12 ml) respectively ($P < 0.0001$). The compliances for the right ear and left ear were lower for the type II diabetic group (0.18 ± 0.05 ml) and (0.18 ± 0.05 ml) compared to the control group (0.28 ± 0.19 ml) and (0.28 ± 0.49 ml) ($P < 0.0001$) respectively. In conclusion, control rats exhibited type A tympanograms with a highly functional middle ear system. Diabetic type I rats (n = 20) mostly exhibited type B tympanograms with a less compliant middle ear system. Compliance was reduced in the diabetic type I and II animals compared to the control. Future studies should utilise histological methods alongside tympanometry. Sections of the middle ear could be used to analyze ossicle size and confirm size differences. This information would be useful in avenues for treatment options for hearing loss in diabetes.

Keywords

Diabetes, Tympanometry, Auditory Function, Compliance, Ear Canal

1. Introduction

Diabetes mellitus is characterized by hyperglycemia resulting from abnormality of insulin secretion, tissue resistance to insulin's actions or both. In type I diabetes insulin insufficiency is due to auto-immune mediated destruction of pancreatic β-cells. In type 2 diabetes, there are varying severities of insulin resistance and relative insulin deficiency. People at risk of type II diabetes are typically obese hypertensive and dyslipidemic [1].

Hearing impairment affects over two-thirds of adults with diabetes, approximately double that of non-diabetic adults. However, there is a lack of information regarding the nature of this impairment [2]. There are three categories of hearing loss: conductive, sensorineural, and mixed hearing loss [3]. Furthermore, there is a wide range of data on the association between diabetes and sensorineural hearing loss, including morphological changes in the number of cochlea receptors among diabetic I and II patients [4].

Whether there is a link between diabetes and conductive hearing loss is debatable. For example, it has been reported that the incidence of conductive hearing loss is 23% in diabetic patients without any history of middle ear disease [5]. However, hyperglycemia may induce damage to small blood vessels in the ear in the same way as it causes retinopathy and damage to kidney blood vessels [6]. In addition, conductive deafness may be due to the thickening of the basement membrane as a result of hypoxia [7], reduced immunity, recurrent middle ear infection, and degeneration and necrosis of the small ear bones [8]. Hyperglycemia is linked to impaired oxidative phosphorylation and reduced ATP availability for high-energy demand structures such as the stria vascularis [9] [10]. Another potential mechanism involves the interaction of advanced glycation end-products (AGEs) and their cellular receptors (RAGEs), resulting in recruitment of inflammatory cells, subsequent endothelial dysfunction, generation of reactive oxygen species (ROS), and ultimately vascular complications of diabetes [11]. RAGE expression has also been identified in regions of the rat cochlea such as the organ of Corti, spiral ganglion, and stria vascularis [11]. Oxidative stress induced by ROS may play a pivotal role in the inner-ear systems [12] [13]. Finally, microangiopathy has been implicated in diabetes-related hearing deficiency at low to high frequencies [14].

Tympanometry can be used to study and compare characteristics of middle ear function; the output is a graph known as a "tympanogram". This technique determines the acoustic admittance of the middle ear, based on air pressure changes in the external auditory canal [15]. A "test tone" of 226 Hz is effective for identifying middle ear disorders. Auditory brainstem response (ABR) has been used to evaluate hearing loss in rodents [16] but the results do necessarily indicate a middle ear infection and vice versa. Tympanometry is considered superior for diagnosing middle ear lesions.

Animal models are a useful way of capturing the diversity of changes observed in diabetic patients. The aims of this study are to study and compare tym-

panometry variables between streptozotocin-induced rats, a model of diabetes mellitus type I and Zucker rats, a model of type II diabetes.

2. Material and Methods

2.1. Animals

Type 1 diabetic rats. Male Sprague Dawley rats (n = 40) aged 5 - 7 weeks (150 - 200 g) were housed in pairs on a 12 h light: 12 h dark cycle with free access to food and water. We did not include female rats because they are less sensitive to streptozotocin. For diabetes induction, rats (n = 20) were fasted for 4 - 6 h before administration of a single i.p. bolus injection of 100 mg/kg streptozotocin (STZ) (S-0130, Sigma, UK; dissolved in sodium citrate; pH 4.5). Seven days following the STZ, blood glucose was measured in a sample of venous blood (obtained by tail prick) taken from each animal (OneTouch® Ultra® 2; Lifescan, Inc., USA). All the STZ-treated animals had a blood glucose of >15 mmol/L (280 mg/dL).

Type 2 diabetic rats. Ten weeks old male Zucker fatty diabetes mellitus (ZFDM) (Hos: ZFDM-fa/fa) rats (300 - 350 g) (n = 20) and their lean littermates (Hos: ZFDM-Lean (fa/+) (200 - 250 g) (n = 20) were housed in pairs; conditions were identical to those of the type 1 diabetic rats. The ZFDM-fa/fa animals obtain non-fasting blood glucose of 300 mg/dL) as early as 10 weeks of age, with a 100% cumulative incidence of diabetes by 21 weeks [17].

2.2. Protocol

This study was approved by the Research Ethics Committee of the Arabian Gulf University and RCSI medical university Bahrain, under project titled Diabetes type I and Middle Ear Pathology May 22nd, 2017; Grant number AGU/RCSI 2017-2018.

2.3. Tympanometry Procedure

Tympanometry measurements were conducted in a quiet room, using a MT 10 tympanometer (Interacoustics, Assens, Denmark). Physical volumes (1.5 ml, 0.5 ml, and 0.25 ml) of the tympanometer were recorded, and measurements were taken from each ear.

2.4. Tympanometry Variables

The MT 10 measures canal volume (V in ml) [18], middle ear pressure (P in daPa), gradient (in ml) which is the steepness of the slope near the peak of the tympanogram [19], and compliance volume which is the greatest amount of acoustic energy absorbed by the middle ear and corresponds to the the vertical peak of the tympanic tracing [20].

2.5. Tympanometry Classification

The classification of tympanograms has been well characterised elsewhere for humans and rodents [20] [21]. Briefly, a "type A" tracing indicates a normal ear

and is described a "bell-shaped curve with peak admittance occuring at or near zero data". A "type B" tracing is abnormal and indicates that the middle ear is filled with fluid and the tympanum is rigid; it is described as a flat curve. A "type C" curve is an unreliable indicator of pathology but may be useful when correlated with other data; it is characterised by a bell-shaped curve in the negative pressure range. When the pressure is between −100 and −199 daPa it is called a type C1 curve and when between −200 and −400 a type C2 curve. Finally, if no peak is detected on the trace then this is termed a "NPF".

2.6. Data Analysis

This data was analyzed using Microsoft Excel 2016. Descriptive statistics was used to analyze continuous and categorical data. This was presented in the form of frequencies in graphs and as means ± SEM unless otherwise noted. An online T calculator was used to confirm differences within and between groups for the tympanometry variables. The level of significance was fixed at $P < 0.05$.

3. Results

3.1. Group Comparisons

Control animals had normal type A tympanograms. Twenty one (75%) of the tympanic membranes in the type 1 diabetic group had abnormal tympanograms of which 46% were type b, 28% had no peak found, and 1% were type c ($P < 0.05$) (Figure 1).

3.2. Between Group Comparisons

The compliances for the right ear and left ear were lower for the type I diabetic group (0.17 ± 11 ml) and (0.13 ± 0.12 ml) compared to the control group (0.20 ± 0.11 ml) and (0.18 ± 0.15 ml) ($P < 0.0001$) respectively.

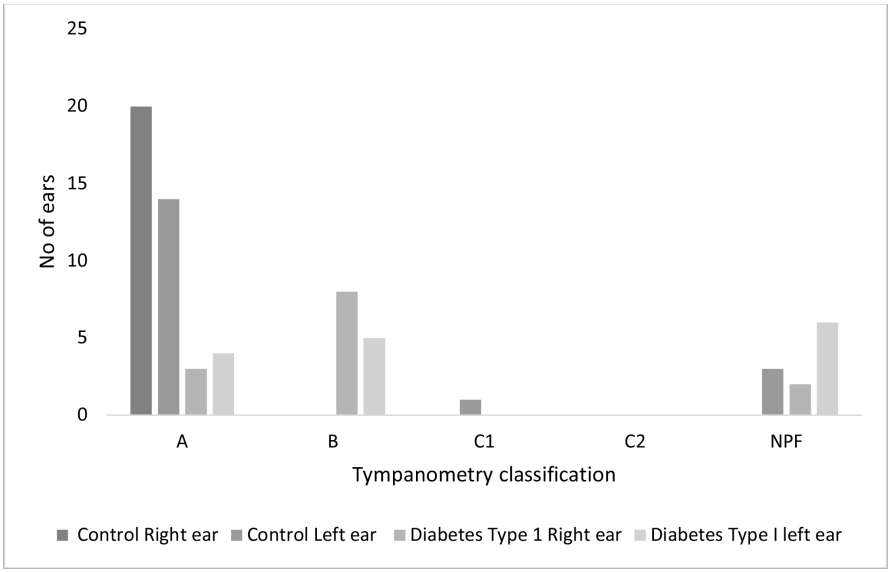

Figure 1. Tympanometry classification of control rats versus diabetic type I rats.

The compliances for the right ear and left ear were lower for the type II diabetic group (0.18 ± 0.05) and (0.18 ± 0.05 dap) compared to the control group (0.28 ± 0.19) and (0.28 ± 0.49) ($P < 0.0001$) respectively (Table 1).

Ear canal volume measurements between the control group and diabetic groups showed a significant difference in terms of being higher for the right ear for the type I (0.22 ± 0.08 ml) and lower for the type II mice (0.15 ± 0.1 ml) compared to the control of (0.17 ± 0.19 ml) and (0.26 ± 0.15 ml) ($P < 0.0001$) respectively. The ear canal measurements were lower in the left ear in type I mice (0.19 ± 0.07) and higher in the left ear for type II mice (0.23 ± 0.15 ml) compared to the controls of (0.39 ± 0.14 ml) and (0.2 ± 0.12 ml) respectively ($P < 0.0001$) (Table 2).

Gradient measurements between the control group and the diabetic type I group showed a decrease for the right ear with values of (127.80 ± 0.17 ml) and (115.38 ± 0.08 ml) ($P > 0.0001$) respectively. Gradient measurements between the control group and the diabetic type I group showed an increase for the left ear with values of (125 ± 91 ml) and (160.78 ± 113 ml) respectively.

Gradient measurements between the control group and the diabetic type II group showed a decrease for the right ear with values of (101 ± 91 ml) and (44.5 ± 41.9 ml) respectively. Gradient measurements between the control group and the diabetic type II group showed an increase for the left ear with values of (24 ± 26 ml) and (54 ± 61 ml) ($P > 0.0001$) respectively (Table 3).

The pressures in the right ear were lower for the type I diabetic group (−40.92

Table 1. Compliance measurements in control versus experimental diabetes animals. Compliance is in ml.

Ear/Compliance in ml	Right	Left
Control	0.20 ± 0.11	0.18 ± 0.15
Diabetes Type I	0.17 ± 0.13	0.13 ± 0.12
Control Hos: ZFDM-Lean (fa/+)	0.28 ± 0.19*	0.28 ± 0.49*
Diabetes Type II Hos: ZFDM-(fa/fa)	0.18 ± 0.05*	0.18 ± 0.05*

Table 2. Ear Canal measurements in control versus experimental diabetes animals. Ear canal volumes in ml.

Ear/Volume in ml	Right	Left
Control	0.17 ± 0.19*	0.39 ± 0.14*
Diabetes Type I	0.22 ± 0.08*	0.19 ± 0.07*
Control Hos: ZFDM-Lean (fa/+)	0.26 ± 0.15*	0.2 ± 0.12*
Diabetes Type II Hos: ZFDM-(fa/fa)	0.15 ± 0.1*	0.23 ± 0.15 *

Table 3. Gradient measurements in control versus experimental diabetes animals. Gradient is in ml.

Ear/Gradient in ml	Right	Left
Control	127.80 ± 0.17	125 ± 91
Diabetes Type I	115.38 ± 0.08	160.78 ± 113
Control Hos: ZFDM-Lean (fa/+)	101 ± 91	24 ± 26
Diabetes Type II Hos: ZFDM-(fa/fa)	44.5 ± 41.9	54 ± 61

Table 4. Pressure measurements in control versus experimental diabetes animals. Pressure is in.

Ear/Pressure in daPa	Right	Left
Control	9.85 ± 50	8.17 ± 46.9
Diabetes Type I	−40.92 ± 87	26 ± 44
Control Hos: ZFDM-Lean (fa/+)	−52 ± 78	−95 ± 141
Diabetes Type II Hos: ZFDM-(fa/fa)	30.8 ± 189	151.5 ± 47.3

Data are expressed as mean ± SD. N = 20 control rats. N = 20 Diabetic type 1 rats. N = 20 Hos: ZFDM-Lean (fa/+). N = 20 Hos: ZFDM-(fa/fa) * = ($P < 0.0001$).

± 87 daPA) compared to the control group (9.85 ± 50 daPa) and higher for the left ear (26 ± 44 daPa) compared to the control group (8.17 ± 46.9 daPa) (Table 4). The pressures in the right and left ear were higher for the diabetic type II mice compared to the controls (30.8 ± 189) and (151.5 ± 47.3 daPa) versus (−52 ± 78) and (−95 ± 141) daPa respectively (Table 4).

4. Discussion

In this study, control rats exhibited type A tympanograms with a highly functional middle ear system. Diabetic type I rats mainly exhibited type B tympanograms with a less compliant middle ear system. Compliance was also reduced in the diabetic type I and II animals compared to controls.

4.1. Use of Tympanometry

Tympanometry provides a quick examination of the middle ear. The standard deviations for compliance in our control rats (0.13 - 0.17) are consistent with those reported for humans (0.097 - 0.107 ml) [22] [23] taking into consideration that human equivalent ear canal volume is much bigger than that of rats.

4.2. Tympanometry Classification

Type A tympanograms were higher among the control animals indicating a fully

compliant hearing system, and the B tympanograms were higher among the diabetic type I group suggesting fluid or infection in the middle ear although this cannot be confirmed) [22] [24].

Type B mean tympanogram amplitudes in the diabetic type 1 rats were significantly lower in both ears than those of control rats. This coincides with a similar study where tympanograms were studied in patients with type 1 diabetes and control subjects (20 - 40 years old) where reduced mean amplitudes were noted [23]. In one study, the associated hearing impairments to diabetes type II were associated with diabetic neuropathy [25].

4.3. Tympanometry Parameters: Compliance

We observed a reduction in compliance of the right ear of type 1 diabetic rats versus controls. There are several possible explanations for this observed reduction in compliance of the tympanic membrane including: 1) weakness of elastin and collagen fibers in the tympanic membrane and/or the ligaments of the ossicular system; 2) inflammation in the middle ear; 3) disorders affecting the cartilage or ossicles of the middle ear [26]. Additional studies are required to confirm these possibilities. Additonally, we noted an increased compliance in the left ear of type 1 diabetic rats compared to the controls. This phenomenon has been noted in children suffering with recurrent acute otitis media [27]. However, we are not able to confirm the reasons for the differences between left and right ears.

By comparison, we observed a decrease in compliance of both the left and right ear of the type II diabetic rats compared to the controls. It has been reported that decreased compliance is associated with abnormalities [21]. Our results may indicate a lesser severity of type II diabetes, however this cannot be confirmed. However, others have concluded that type II diabetes is more severe in terms of hearing loss at high frequencies [25]. Possible reasons for this more severe effect include lower serum levels of protein oxidation products, nitric oxide, and antioxidants activity [28].

4.4. Tympanometry Parameters: Pressure and Canal Volume

Interestingly, we found lower readings for the ear canal volume and pressure on the left compared to the right side in both types of diabetic animals. This agrees with one study which highlighted a relationship between type 1 diabetes and severity of left sided hearing loss with longer disease length [29]. In addition, hearing loss may be more related to glycaemic control rather than diabetes *per se* [30]. Further studies could look at the (HbA1c) levels in the two groups.

4.5. Limitations

Performing an airtight seal between the tympanometer and the ear canal was not always straightforward. We had some success by changing the tip size and gently pulling the external ear up to stretch the ear canal. However, the standard deviations of our measurements indicate that the problem was minimized. Our results

could have been strengthened by including middle ear histology however were unable to do so due to technical limitations.

5. Conclusions

In conclusion, control rats exhibited type A tympanograms with a highly functional middle ear system. Diabetic type I rat mostly exhibited type B tympanograms with a less compliant middle ear system suggesting fluid or infection in the middle ear. Compliance was reduced in the diabetic type I and II animals compared to the control.

Future studies could look at a sophisticated animal study using several measurement techniques including histology. Sections of the middle ear could be used to analyze ossicle size and confirm size differences.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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