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# ORIGINAL ARTICLE

## AUDIOLOGICAL PROFILE IN DIABETES MELLITUS IN CORRELATION WITH INFLAMMATORY MARKERS

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**ABSTRACT: OBJECTIVE:** To correlate the audio-logical profile in diabetes mellitus with inflammatory markers (TNFα, IL-6, CRP, and FREE RAICALS). **MATERIALS AND METHODS:** A two year prospective study of Audio-logical profile and its correlation with inflammatory markers was done in 35 diabetic patients attending Diabetic OPD and ENT OPD at RMMCH between 2012-2014.Patients with diabetes mellitus on oral hypoglycaemic agents or insulin therapy was subjected to Pure Tone Audiometry using Arphi Audiometer-Model 700MK4 and Inflammatory marker study. **STATISTICS AND ANALYSIS:** P-value was found out by ANOVA method to find out the significance of auditory thresholds between various categories of parameters. **RESULTS:** Patients with Diabetes Mellitus were more commonly affected by Sensori-neural Hearing loss in the age group between 41-49 years accounting for 77.14%. Females were more affected in our study in the ratio3.3:1. Hearing loss was bilaterally symmetrical and of sensori-neural type affecting mainly the higher frequencies. In 77.1% of our patients the duration of diabetes mellitus was less than 5years, however there was no correlation between the duration of diabetes mellitus and hearing loss. Control in the severity of diabetes mellitus lead to improvement in hearing thresholds, which was statistically significant at higher frequencies. **CONCLUSION:** There was a positive correlation between increased level of inflammatory markers-TNF α, IL-6, CRP, free radicals and hearing loss in diabetes mellitus. However the inclusion of elevated markers as a prognostic index to assess the degree of deafness and its relation to microangiopathy needs further studies and its role in prevention of deafness in diabetes mellitus needs further evaluation.

**KEYWORDS:** Audio-logical Profile, Diabetes Mellitus, Inflammatory Markers, TNF α, IL-6, CRP, Sensori-neural Hearing Loss, Free Radicals.

**INTRODUCTION:** Diabetes mellitus is a common medical disorder world-wide. Diabetes is derived from the Greek WORD "siphon" and implies that lot of urine is made. The second term "mellitus" comes from the Latin word Mel which means honey, and was used because the urine was sweet. World-wide around 150 million people suffer from diabetes mellitus and without preventive measures it is expected to reach 300 million by the year 2025.

Diabetes mellitus is a medical disease characterized by high blood sugar levels that result from defective insulin secretion, or action, or both. Normally blood glucose levels are tightly controlled by insulin, a hormone produced by the beta cells in the islets of Langerhans in the pancreas. Insulin lowers the blood glucose levels. When the blood glucose elevates, insulin is released from the pancreas to normalize the glucose levels. In patients with diabetes, the absence or insufficient production of insulin causes hyperglycemia.

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Diabetes mellitus is of two types-types 1 and Type 2. In type 1 diabetes mellitus the beta cells degenerate and so the body cannot make enough insulin of its own. In type 2 diabetes mellitus beta cells produce insulin but there is resistance in cells to insulin. Diabetes is a chronic medical condition, meaning that although it can be controlled, it lasts a life time. Over time, diabetes can lead to retinopathy, nephropathy and neuropathy.

This type of damage is the result of injury to smaller vessels, referred to as Micro vascular Disease. Diabetes is also an important factor in accelerating the hardening and narrowing of arteries –Atherosclerosis leading to strokes, coronary artery disease, and other large blood vessel disorder, referred to as Macro-Vascular disease. The Cochlea and Auditory nerve can also be affected by raised blood sugar levels, possibly a micro vascular complication of diabetes. Sensorineural hearing loss accounts for about 90%of all hearing loss.

This is sometime also called Nerve Deafness although the term is not entirely accurate, leaving out disorders of the hair cells of the cochlea. The relationship between diabetes mellitus and hearing loss has been debated for many years. Jordao in 1857 reported a case of diabetic patient with hearing loss[1,2,3] and Edgar in 1915[4] was the first to report a high frequency hearing loss in a diabetic patient.

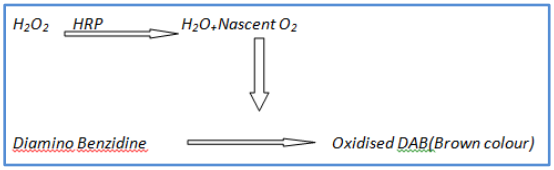
**MATERIALS AND METHODS:** A 2 year prospective study of audio-logical profile in diabetic patients and its correlation with inflammatory markers was done in 35 diabetic patients attending the Diabetic and ENT OPD at RMMCH between 2012-2014. Patients with diabetes mellitus on oral hypoglycaemic agents were subjected to: Pure Tone Audiometry using Arphi Audiometer-Model 700 MK4 and Inflammatory markers study.

**INCLUSION CRITERIA:** All patients with type 2 diabetes mellitus on insulin or oral hypoglycaemic drugs between the ages of 15-50 years.

**EXCLUSION CRITERIA:** Previous history of ear discharge, noise trauma and recurrent upper respiratory tract infection and conductive hearing loss. Patients were followed up serially after the diagnosis at 1st, 2nd, 3rd, and 4th and 6 months for control of diabetic status and their audio-logical profile. The same was correlated with the inflammatory markers. The results were statistically analyzed. The audiograms were also analyzed by an outside audio-metrician not involved in the study to overcome bias in results. Inflammatory marker study was done by ELISA method.

ELISA Test: ELISA is the abbreviation for Enzyme Linked Immuno Sorbent Assay. The ELISA technique are widely used not only for hormone measurements but also for detecting growth factors, tumor markers, bacterial or viral antigens and antibodies against microbes and other antigens or antibodies in biological fluids. Antigen Detection by ELISA: Specific antibody is fixed to the wall of micro titer plate. The patient's serum is added to the well, and incubated for 30minutes at 37°C.

By the time, if the serum contains antigen, it is fixed on the antibody. Excess antigen and other unwanted proteins are washed out. Then specific antigen tagged with horse- radish peroxidase is added. If the antigen is already fixed, the antibody. HRP conjugate will be fixed in the well. Then the colour reagent containing hydrogen peroxidase ($H_2O_2$) and diaminobenzidine (DAB) are added.



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This is known as "SANDWICH ELISA". Development of a brown colour indicates that the antigen is originally present in patient's serum. Therefore intensity of the colour may be measured, from which the concentration of antigen is calculated.

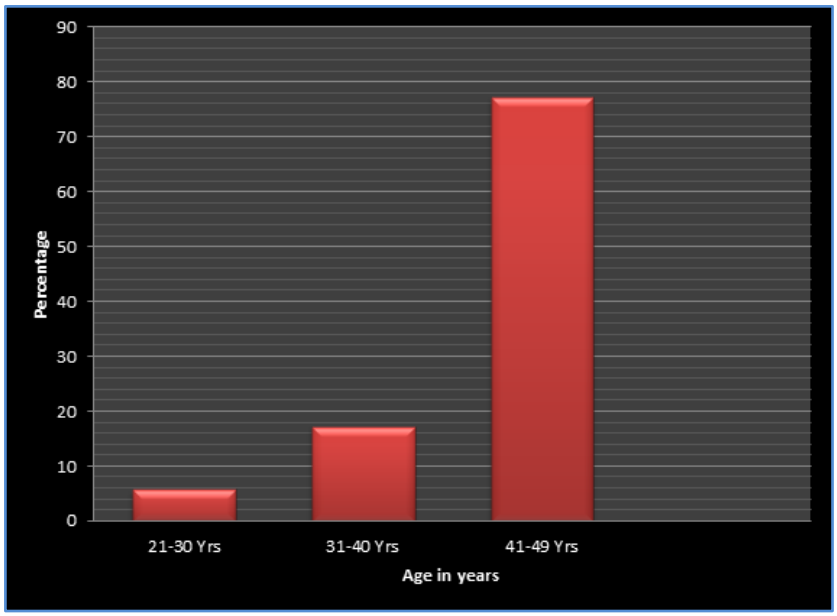
**STATISTICAL METHODS:** P-value was found out by ANOVA method to find out the significance of auditory thresholds between various categories of parameters.

**RESULTS:**

| Age | Number of Patients | Percentage |
|---|---|---|
| 21 to 30 Years | 2 | 5.71 |
| 31 to 40 Years | 6 | 17.14 |
| 41 to 49 Years | 27 | 77.14 |
| **TOTAL** | **35** | **100.0** |

Table 1: Distribution of age

In this study the maximum number of patients with hearing loss was in the age group between 41-49 accounting for 77.14%.




| Gender | Number of patients | Percentage |
|---|---|---|
| Male | 8 | 22.86 |
| Female | 27 | 77.14 |
| **TOTAL** | **35** | **100** |

Table 2: Distribution of gender

Females were more affected by hearing loss in our study accounting for77.14%.

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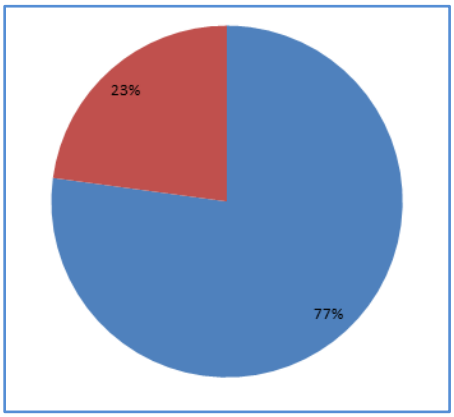


| Type of hearing loss | Number of low frequency loss | Number Of Mid frequency loss | Number Of High frequency loss |
|---|---|---|---|
| Sensorineural hearing loss B/L | 5 | 30 | 35 |
| Mixed hearing loss B/L | 30 | 5 | - |
| **TOTAL** | **35** | **35** | **35** |

Table 3: Type of hearing loss

In our study the hearing loss in diabetes mellitus was predominantly bilateral, symmetrical, sensor neural type hearing loss affecting the higher frequencies.

| Duration of diabetes mellitus | Number of patients | Percentage |
|---|---|---|
| 0-5 Years | 27 | 77.1 |
| 6-10 Years | 7 | 20 |
| 11-15 Years | 0 | 0 |
| Above 15 Years | 1 | 2.9 |
| **TOTAL** | **35** | **100** |

Table 4: Distribution of duration of diabetes mellitus

In 77.14% of our patients the duration of diabetes mellitus from diagnosis was less than 5 years.

| Frequency in Hertz | Duration of diabetes mellitus with hearing loss in decibels | | | | P value (ANOVA) |
|---|---|---|---|---|---|
| | 0-5 Years (n=27) | 6-10 Years (n=7) | 11-15 Years (n=0) | Above 15 Years (n=1) | |
| 500 | 54.21±11.46 | 53.44±15.64 | - | 53.64±0.00 | 0.264(NS) |
| 1000 | 59.42±14.62 | 59.12±13.63 | - | 58.94±0.00 | 0.642(NS) |

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| | | | | | |
|---|---|---|---|---|---|
| 2000 | 62.64±13.54 | 61.59±11.48 | - | 62.16±0.00 | 0.112(NS) |
| 3000 | 56.34±12.37 | 55.36±12.56 | - | 67.50±0.00 | 0.658(NS) |
| 4000 | 60.00±13.81 | 60.64±13.81 | - | 70.00±0.00 | 0.269(NS) |
| 6000 | 62.96±15.29 | 62.50±15.86 | - | 85.00±0.00 | 1.006(NS) |
| 8000 | 64.72±16.18 | 64.46±17.59 | - | 82.50±0.00 | 0.571(NS) |

Table 5: Duration of diabetes mellitus with hearing loss

NS-Not Significant P>0.05 S-Significant P <0.05. The duration of diabetes mellitus is compared with the hearing loss in db in all frequencies and is not statistically significant (P>0.05 not significant by ANOVA method)

| **Frequency in Hertz** | **Severity of diabetes mellitus with hearing loss in db (Mean ± SD)** | | | | **P value by ANOVA** |
|---|---|---|---|---|---|
| | **50-100mg/ dl (n=5)** | **101-150mg/dl (n=18)** | **151-200mg/ dl (n=5)** | **Above200mg/ dl (n=7)** | |
| 500 | - | 54.21±11.64 | 54.62±12.45 | 54.55±12.65 | 0.612(NS) |
| 1000 | - | 55.64±10.21 | 55.16±11.64 | 55.62±11.22 | 0.882(NS) |
| 2000 | - | 56.94±13.54 | 56.26±12.11 | 56.33±12.64 | 0.112(NS) |
| 3000 | - | 57.50±12.59 | 59.75±12.61 | 58.13±9.23 | 0.894(NS) |
| 4000 | - | 61.18±15.49 | 59.75±12.61 | 58.13±9.23 | 0.910(NS) |
| 6000 | - | 65.29±16.88 | 68.75±16.13 | 63.44±14.45 | 0.773(NS) |
| 8000 | - | 67.65±15.99 | 68.00±17.39 | 65.94±17.83 | 0.962(NS) |

Table 6: Auditory thresholds in Decibels at various levels of FBS-1$^{ST}$Month

NS- Not Significant P >0.05 S-Significant (P<0.05)

| **Frequency** | **Severity of diabetes mellitus with hearing loss in decibels** | | | | **P value by ANOVA** |
|---|---|---|---|---|---|
| | **100-150mg/ dl(n=6)** | **151-200mg/ dl(n=1)** | **201-250mg/ dl(n=2)** | **Above250mg/ dl (n=9)** | |
| 500 | 55.31±9.11 | 55.64±10.32 | 55.21±11.62 | 55.11±10.22 | 0.321(NS) |
| 1000 | 56.64±10.34 | 56.21±11.12 | 56.11±11.26 | 56.64±10.11 | 0.642(NS) |
| 2000 | 57.24±9.64 | 57.64±9.66 | 57.99±10.42 | 57.99±10.64 | 0.112(NS) |
| 3000 | 56.25±9.97 | 52.73±9.65 | 61.94±14.57 | 62.78±10.03 | 0.177(NS) |
| 4000 | 60.00±10.95 | 55.23±10.98 | 64.72±18.22 | 64.72±16.02 | 0.395(NS) |
| 6000 | 62.92±9.54 | 60.23±12.77 | 71.39±19.41 | 69.17±18.11 | 0.390(NS) |
| 8000 | 65.42±10.54 | 62.05±10.17 | 72.22±20.67 | 70.28±20.71 | 0.524(NS) |

Table 7: Auditory thresholds in decibels at various levels of PPBS 1$^{st}$ Month

In the first month auditory thresholds at all frequencies had no correlation with severity of diabetes in both fasting and post prandial blood sugar levels(P>0.05 not significant by ANOVA method).

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| Frequency in Hertz | Severity of diabetes mellitus with hearing loss in decibels | | | | P value by ANOVA |
|---|---|---|---|---|---|
| | 50-100mg/ dl(n=5) | 101-150mg/ dl(n=18) | 151-200mg/ dl(n=7) | Above 200mg/ dl(n=7) | |
| 500 | 61.61±10.34 | 61.22±9.97 | 61.49±10.99 | 62.64±11.14 | 0.64(NS) |
| 1000 | 59.98±9.98 | 59.79±9.78 | 59.97±10.64 | 59.12±10.11 | 0.421(NS) |
| 2000 | 60.64±10.64 | 61.14±10.11 | 62.34±9.64 | 60.21±9.12 | 0.342(NS) |
| 3000 | 59.50±11.24 | 62.36±14.69 | 70.00±18.37 | 76.43±16.76 | 0.159(NS) |
| 4000 | 61.50±13.18 | 63.47±13.62 | 69.50±18.91 | 80.00±19.15 | 0.010(S) |
| 6000 | 52.00±12.55 | 54.72±11.08 | 65.00±8.48 | 67.14±8.09 | 0.013(S) |
| 8000 | 55.00±13.69 | 56.53±12.55 | 67.00±14.40 | 72.14±13.18 | 0.041(S) |

Table 8: Auditory thresholds in decibels at various levels of FBS 6thmonth

| Frequency in Hertz | Severity of diabetes mellitus with hearing loss in db(Mean ± SD) | | | | P value by ANOVA |
|---|---|---|---|---|---|
| | 100-150mg/ dl(n=6) | 151-200mg/ dl(n=11) | 201-250mg/ dl(n=9) | Above250mg / dl (n=9) | |
| 500 | 54.34±11.21 | 55.21±10.42 | 55.64±9.64 | 55.21±10.36 | 0.242(NS) |
| 1000 | 55.36±9.64 | 55.14±9.24 | 56.64±10.31 | 56.11±11.45 | 0.452(NS) |
| 2000 | 56.33±11.64 | 55.31±10.22 | 55.11±9.64 | 55.64±10.42 | 0.642(NS) |
| 3000 | 57.00±10.59 | 58.75±11.26 | 55.91±14.46 | 64.17±8.01 | 0.565(NS) |
| 4000 | 62.50±11.84 | 59.69±14.54 | 58.64±17.80 | 64.17±12.81 | 0.014(S) |
| 6000 | 65.75±10.93 | 64.06±16.03 | 64.55±19.13 | 70.83±18.82 | 0.012(S) |
| 8000 | 64.50±12.36 | 67.19±16.00 | 66.27±20.01 | 67.50±19.43 | 0.044(S) |

Table 9: Auditory thresholds in decibels at various levels of PPBS-6th Month

NS- Not Significant P>0.05 S-Significant. After 6 months of treatment the auditory threshold improved at higher frequencies (4000,6000,8000 Hz) in both fasting and postprandial blood sugar levels (P<0.05 significant by ANOVA method)

| | Number | Diabetes Mean ± SD | Mean Hearing loss in decibels | | | F-Value | P Value |
|---|---|---|---|---|---|---|---|
| | | | | 1st Month | 6th Month | | |
| FBS 1st Month | 35 | 158.14±67.55 | Low | 55.31±16.34 | 55.64±14.64 | 1.642 | 0.642(NS) |
| PPBS 1st Month | 35 | 225.14±93.34 | Mid | 59.41±15.64 | 58.64±15.21 | 1.483 | 0.242(NS) |
| FBS 6th Month | 35 | 159.23±42.96 | High | 64.52±18.84 | 61.46±25.60 | 61.925 | 0.001(S) |
| PPBS 6th Month | 35 | 200.66±61.60 | | | | | |

Table 10: Control of diabetes mellitus and hearing

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S-Significant NS-Non Significant.

P value<0.05 P value 0.05.

The fasting and post prandial blood sugars of 1st and 6th Month. In our study the control of diabetes leading to improvement in hearing thresholds was significant only in high frequencies P value <0.05 by ANOVA method.

**CORRELATION ANALYSIS:**

| | TNF α | IL-6 | CRP | FREE RADICALS/T BARS |
|---|---|---|---|---|
| Hearing loss in all frequencies | 0.033 | 0.095 | 0.135 | 0.019 |

Table 11: Correlation between hearing loss and inflammatory markers

The hearing loss in all frequencies were correlated with the inflammatory markers (TNFα,IL -6,CRP and Free radicals) and a positive relation was found.

| | N | Mean | SD | F-VALUE | P-VALUE |
|---|---|---|---|---|---|
| Hearing loss in all frequencies | 35 | 63.07 | 24.20 | 45.711 | 0.001(S) |
| TNF α | 35 | 12.51 | 23.31 | | |
| IL-6 | 35 | 40.04 | 36.84 | | |
| CRP | 35 | 6.90 | 8.84 | | |
| Free radicals/ T bars | 35 | 2.17 | 1.04 | | |
| **TOTAL** | **175** | **24.94** | **32.26** | | |

Table 12: ANOVA TABLE

Highly Significant P<0.01

The hearing loss in all frequencies is compared with the inflammatory markers and is found to be statistically significant <0.01) by ANOVA method.

**DISCUSSION:** There are various studies that have been done which shows high frequency sensori-neural hearing loss and elevated inflammatory markers (TNFα, IL-6, CRP) to be associated with patients who are having diabetes mellitus. In the present study the maximum number of patients with hearing loss was in the age group between 41-49 years (77.14%) followed by the age group 31-40(17.14) and was comparable with other studies. Females were more affected in the ratio 3.3:1.

The type of hearing loss was predominantly bilateral, symmetrical, and sensori-neural type affecting the higher frequencies in diabetes mellitus. In 77.14% of our patients the duration of diabetes mellitus was less than 5 years. The duration of diabetes mellitus had no significant correlation with hearing loss (P>0.05-NS). In the present study, control of diabetes mellitus evidenced by reduced FBS and PPBS levels lead to improvement in hearing thresholds and was significant only in high frequencies (4000, 6000, 8000Hz) (P<0.05).

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In our study of 35 patients, we found that there is significant increase in inflammatory markers in all cases ($p \geq 0.01$) and a positive correlation was found between inflammatory markers and hearing loss. Free radicals are not so significantly increased when compared to inflammatory markers in cases of diabetes associated with deafness.

**SUMMARY AND CONCLUSION:** Sensori-neural hearing loss in diabetes mellitus more commonly affected patients in the age group between 41-49 years accounting for 77.14%.Females were more affected in our study in the ratio of 3.3:1.Hearing loss was predominantly bilaterally symmetrical and of sensori-neural type affecting mainly the higher frequencies. In 77.1% of our patients the duration of diabetes mellitus was less than 5 years. However there was no correlation between duration of diabetes mellitus and hearing loss.

Control in the severity of diabetes mellitus lead to improvement in hearing thresholds which was spastically significant at higher frequencies. There was positive correlation between inflammatory markers and hearing loss in diabetes mellitus however inclusion of elevated inflammatory markers as a prognostic index to assess the degree of deafness and its relation to microangiopathy needs further studies and its role in prevention of diabetes mellitus needs further evaluation.

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