

Research Article

Effects of Rh-Negative Blood Group on Pure Tone Thresholds and Immittance Test Results

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Short running title: Effects of Rh-Negative Blood Group on Pure...

Highlights:

- Pure tone thresholds and immittance results were compared between blood groups
- There is an elevation of acoustic reflex thresholds for persons with blood group O
- There is no difference in pure tone thresholds and tympanometric findings

ABSTRACT

Background and Aim: Human blood group system variations may impact the individual's health and hearing status. It was found that individuals with O-positive blood are more prone to noise-induced hearing loss, and they exhibit reduced amplitude in otoacoustic emissions, elevated Acoustic Reflex Threshold (ART), and a slightly higher resonance frequency of the middle ear. Hence, a study was needed to observe if the same trend is followed for those with Rh-ve blood groups.

Methods: A cross-sectional group post-test-only design with a non-random convenient sample was applied. This study was conducted with forty adult female participants with a pure tone threshold range of less than or equal to 15 dBHL and ages ranging from 18 to 25 years. In each blood group (A-, B-, O-, AB-), 10 participants were recruited. All participants underwent immittance evaluation, pure tone audiometry, and extended high-frequency audiometry.

Results: On analyzing the data, participants with the O negative blood group showed significantly elevated ART in both ipsilateral and contralateral ears. Also, there was no significant difference for pure tone thresholds for the frequencies 250 Hz to 16 kHz and tympanometry findings.

Conclusion: This study indicates that the elevated ARTs in O negative blood group people could be attributed to relatively increased stiffness in the middle ear and fewer outer hair cells compared with other blood groups.

Keywords: Negative blood group; acoustic reflex thresholds; outer hair cells; noise-induced hearing loss

Introduction

The ABO blood group system with the phenotypes including A, B, AB, and O were established. The uniqueness of the ABO blood group system is that it is the only human antigen system in which antibodies develop naturally against non-native antigens [1]. The Rh blood group system, which consists of at least 45 distinct antigens and is the most polymorphic of all human blood groups, is among the most therapeutically significant in transfusion medicine, second only to ABO [2]. Red Blood Cells' (RBCs) surface contains a genetically inherited protein called Rh factor, sometimes called the "Rhesus factor." For practical purposes, there are just two blood groups – Rhesus positive and Rhesus negative– despite the complicated genetic underpinnings. An individual's RBCs are classified as Rh positive or Rh negative depending on whether the Rh protein is present or absent [1].

The differences in the human blood group system can affect the health and hearing status –increasing susceptibility to acquiring a particular disorder due to the presence or absence of specific antigens on the surface of RBCs [3]. Diseases, including cancerous, infectious, non-infectious, bacterial, and viral infections, are associated with the ABO and Rh blood group systems [4]. Blood-type antigens are momentarily expressed in cochlear hair cells in development. As a result, it may be crucial for the synaptic link between cochlear hair cells, auditory nerve fibers, and neural responses to function properly [5].

Studies have also reported statistically significant changes in Otoacoustic Emissions (OAEs), with reduced amplitude for individuals with blood group O, irrespective of gender [6-8]. In contrast to previous studies, a recent study with a large sample size reveals no significant difference in physiological measures such as OAEs across the blood groups [9]. Few studies have explored the association of the ABO blood group and auditory brainstem responses and reported that the trend is being followed in individuals with the O blood group for wave I with lower amplitude and prolongation of wave I latency. They stated that a person with blood group O is prone to have decreased cochlear/auditory nerve functioning [10, 11]. Studies also investigated the association between Rh antigen and hearing loss. They reported no significant difference between Rh antigen and hearing loss in adults and younger babies [12, 13].

According to earlier research, ABO blood group variations have been linked to hearing loss, particularly Noise-Induced Hearing Loss (NIHL). Compared to other blood groups, those with the O blood group are more likely to acquire NIHL [14]. According to Doğru et al., this might result from fewer healthy outer hair cells [14]. Studies have also reported the effect of the positive blood group on middle ear measures [15, 16].

Prabhu et al. [16] reported elevated Acoustic Reflex Threshold (ARTs) and increased middle ear resonant frequency for persons with an O-positive blood group. Also, researchers inferred this could be due to the relatively increased stiffness and sub-clinical middle ear pathology in persons with positive blood group O. They recommended audiological measures in persons with different blood groups [16].

Based on earlier research, it is known that ABO blood group status could be associated with auditory function. However, it is unclear whether Rh-negative blood group status impacts the immittance test outcomes. Earlier studies have been focused on determining the relations between the different Rh-positive blood group systems and auditory sensitivity and necessitate the study of audiological evaluations in individuals with Rh-negative antigen [6-11]. However, valid and reliable research studies on the relationship between Rh-negative blood groups and hearing sensitivity still being determined in the literature.

Hence, the aim of the study was to compare the pure tone thresholds (dB HL) and immittance test findings of individuals with different blood groups with Rh-negative antigens.

Methods

Study participants

A cross-sectional group post-test-only design with non-random convenience sampling was applied. Forty female participants of age ranging from 18 to 25 years with ABO blood group were recruited for this study. The inclusion criteria are as follows: participants were aware of their blood groups. Accurate blood group details were obtained from the participant's serological test reports, administered during their academic year. Also, ABO blood group status self-reporting has good specificity and sensitivity [17]. Individuals with a history of significant otological problems, prolonged or frequent loud noise exposure, alcohol intake, smoking, ototoxic drugs, family history of hearing loss, and any other medical history were excluded from the study. The above-mentioned conditions could affect the outcomes of the present study. All the participants had negative rhesus factors (Rh). They were

subdivided into four groups based on the ABO blood group system (A-, B-, O-, AB-). In each blood group, 10 participants were included. Only female participants were included in the study as a part of convenient sampling. The current study did not take skin or eye color into account.

Equipment

The testing room was acoustically treated, and all testing procedures followed a standard methodology with a permissible noise level by ANSI/ASA S3.1-199 (R2013) [18]. A calibrated dual channel diagnostic audiometer, Inventis Piano (Inventis Padova, Italy), with Telephonics Dynamic Headphones (TDH) 39 headphones (Huntington, NY) were used to record the air conduction thresholds (dB HL). Sennheiser High-Definition Audio (HDA) 200 headphones (Wedemark, Germany) to obtain high-frequency thresholds (dB HL). A calibrated immittance audiometer, "Inventis Clarinet" (Inventis Padova, Italy), was used to evaluate middle ear status and acoustic reflexes.

Procedures

Before the audiological evaluation, a thorough medical history was taken from each participant to rule out any history of noise exposure, hearing loss, or otological issues. Participants were first evaluated with otoscopy to rule out outer ear and ear canal anomalies, followed by pure tone audiometry and immittance evaluation. All tests were measured in a randomized manner for the right and left ears.

Air conduction thresholds (dB HL) for the octave frequencies from 250 Hz to 8 kHz were measured in both ears. High-frequency auditory thresholds for the frequencies 8 kHz, 10 kHz, 12.5 kHz, and 16 kHz were measured in both ears, respectively. Furthermore, bone conduction testing for 250, 500, 1000, 2000, and 4000 Hz was done to obtain bone conduction thresholds (dB HL) in both ears. Radioear B-71 bone vibrator was used to determine the bone conduction threshold. A modified Hughson-Westlake [19] approach was used to obtain the thresholds in Pure Tone Audiometry (PTA) and high-frequency audiometry testing.

The immittance testing included tympanometry and acoustic reflexes. During the immittance evaluation, participants were not instructed to swallow or make head or body movements. Tympanometry was performed using the probe tone of 226 Hz at the intensity level of 85 dB SPL to obtain compliance (ml), peak pressure (da Pa), and ear canal volume (ml) in both ears [15]. Acoustic reflex threshold (dB HL) testing was carried out at 500 Hz, 1, 2, and 4 kHz in ipsilateral and contralateral ears using manual mode with 5 dB step size. The intensity level of acoustic stimuli was at least 70 dB above the pure tone threshold and measured at 110 dB HL [15, 20]. The ascending method measured the ART at tympanometric peak pressure. The criterion of change in compliance by 0.02 to 0.03 (ml) was used to determine the ART [20]. The study compared the pure tone thresholds (dB HL) (conventional and high frequency), immittance findings (compliance (ml), ear canal volume (ml), and peak pressure (da Pa)), and ARTs (500 Hz, 1 kHz, 2 kHz, 4 kHz ipsilateral and contralateral, respectively) between individuals with different blood groups with Rh negative antigen.

Statistical analyses

The statistical data analysis was done with IBM Corp.'s Armonk, NY SPSS version 21. The data was analyzed using the "Shapiro-Wilk test of normality," which revealed that the data had a normal distribution among the participants ($p > 0.05$). Thus, parametric tests were run to do further statistical analysis.

Results

Pure tone thresholds and blood group findings

The results for the pure tone thresholds were subjected to a descriptive statistical analysis. The thresholds were similar between the blood groups.

Multivariate analysis of variance (MANOVA) was done to compare the differences in pure tone thresholds across the blood groups. The results showed that there was no statistically significant difference [$F_{(27,210)}=1.48$, $p > 0.05$] across blood groups for pure tone frequencies from 250 Hz to 16 kHz. The mean, median, standard deviation, and range of pure tone thresholds from 250 Hz to 16 kHz across blood groups are shown in Table 1.

Tympanometry and blood group findings

The results for the tympanometry parameters, compliance (ml), ear canal volume (ml), and peak pressure (daPa), were subjected to descriptive statistical analysis. The tympanometry parameters were similar between the blood groups. The mean, median, standard deviation, and range of tympanometry results across blood groups are provided in Table 2.

MANOVA was done to compare the differences in tympanometry parameters across the blood groups. The results showed that there was no statistically significant difference [$F_{(9,228)}=1.54$, $p>0.05$] in tympanometry parameters such as compliance (ml), peak pressure (daPa), and ear canal volume (ml) between the blood groups.

The results for the ARTs at .5, 1, 2, and 4 kHz in ipsilateral and contralateral ears were subjected to a descriptive statistical analysis.

Furthermore, MANOVA results showed there was a statistically significant difference [$F_{(24,213)}=2.49$, $p<0.05$] in ARTs (dB HL) for persons with blood group O. Subsequent post hoc tests showed that the ART at .5, 1, 2, and 4 kHz in ipsilateral and contralateral ears was significantly elevated ($p<0.05$) for blood group O compared to other blood groups. However, the results also showed that there was no significant difference ($p>0.05$) in ARTs (dB HL) between blood groups A, B, and AB. The mean and standard deviation of ipsilateral and contralateral ARTs (dB HL) at different frequencies across blood groups are provided in Figures 1 and 2, respectively.

Discussion

For the first time, the purpose of the study was to assess the effects of the Rh-negative blood group on various audiological findings. The results showed no significant difference in the thresholds of frequencies from 250 Hz to 16 kHz for pure tones among Rh- Rh-negative blood groups. These results are consistent with a study [21] that found no statistically significant differences in pure tone thresholds between blood groups. They hypothesized that the blood group status may not impact pure tone thresholds with the Rh-positive blood group [21]. Similar results were found in our study in individuals with Rh-negative blood group.

In the present study, the tympanometric parameters such as ear canal volume, compliance, and peak pressure were similar across the blood groups. These findings are similar to the previous studies, which reported no significant difference in tympanometric parameters across different blood groups. The study was conducted on those with Rh-positive blood groups, and it was reported that the tympanometric parameters were not influenced by blood groups [16]. Similar findings were observed in our study of individuals with Rh-negative blood group. In this study, the ipsilateral and contralateral ART was significantly elevated in individuals with O positive blood group compared to A, B, and AB. The findings of this study are consistent with those of a previous study, which reported elevated ARTs and increased resonant frequency of the middle ear in persons with O-positive blood group [7]. It is reported that the reduction in the number of outer hair cells in individuals with blood group O could be the possible reason for the elevation in reflex thresholds [22, 23]. This is supported by previous studies, which have also reported reduced ultra-high-frequency OAEs, indicating a reduction in cochlear functioning in persons with blood group O [7-9, 16].

Literature indicates that persons with O blood group have decreased clotting factor due to lower levels of protective glycoproteins, and this could be linked to the reduced cochlear/neural functioning for persons with blood group O [12, 24-27]. Thus, the influence of the number of healthy outer hair cells, levels of protective glycoproteins, cochlear synaptic transmission, and biological differences in blood could be attributed to the elevated ARTs in the persons with O blood group [7-10, 12, 24-27]. The results of the present study are in consensus with similar studies done on the Rh-positive blood group. Therefore, the present study could serve as a baseline to understand the differences in audiological findings in individuals with Rh-negative blood groups.

Limitations of the study and future direction

The present study was carried out in a smaller sample size. The findings can be replicated with a large sample size in the future. The present study considered only female participants, and menstrual status was not accounted for, which might have affected the results. Therefore, more research can be carried out on behavioral and electrophysiological measures in males and females, which would provide insights into detail.

Implications of the study

The present study highlights the differences in acoustic reflexes among individuals with negative blood groups. Elevated acoustic reflexes in individuals with blood group groups should be considered while conducting research on acoustic reflexes. In addition, the study suggests that the blood groups should also be considered while interpreting ART in routine clinical practice. Despite hearing thresholds being no greater than 15 dB HL, the ART could be elevated in persons with specific blood groups.

Conclusion

The study aimed to compare the audiological results of individuals from various blood groups. The findings demonstrated that persons with blood group O had an elevated Acoustic Reflex Threshold (ART) compared to other adults. The following inferences can be drawn from our findings. First, the elevated ART could be due to

reduced healthy outer hair cells, biological blood differences, and relatively increased middle ear stiffness. Second, there may be a greater chance of subclinical middle ear pathology in those with blood group O.

Ethical Considerations

Compliance with ethical guidelines

In the current study, all of the testing procedures were accomplished using a non-invasive technique and adhered to the conditions of the institutional ethical approval committee. The institutional ethical approval committee approved the current study Holy Cross College Ethical Committee (HCC/ERB/EC/PB-03/2023-2024). The test procedures were clearly explained to the participants before testing. Informed consent: Prior informed consent was obtained from the participants regarding their willingness to participate in the study.

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Authors' contributions

KK, SR, DS, JX, ARC: Study design, acquisition of data, drafting the manuscript, interpretation of the results; NJW: Supervision, interpretation of the results, drafting the manuscript, critical revision of the manuscript; PP: Study design, supervision, critical revision of the manuscript and statistical analysis.

Conflict of interest

There are no competing financial interests.

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Table 1. Mean, median, Standard deviation, and range of pure tone thresholds across blood groups (n=20)

Frequency	Blood group	Mean (SD)	Median	Range	
				Minimum	Maximum
250 Hz	A	15.30 (5.70)	15	5	25
	B	14.00 (3.10)	15	10	20
	AB	15.50 (4.60)	15	10	20
	O	16.00 (3.50)	15	10	20
500 Hz	A	15.00 (3.20)	15	10	20
	B	14.50 (4.60)	15	5	20
	AB	14.00 (5.00)	15	5	20
	O	14.80 (2.60)	15	10	20
1000 Hz	A	13.50 (4.30)	15	5	25
	B	12.80 (5.00)	15	5	20
	AB	12.50 (5.50)	15	5	20
	O	12.80 (3.00)	12.5	10	20
2000 Hz	A	13.00 (3.40)	15	5	20
	B	13.00 (5.00)	13	0	20
	AB	13.80 (5.10)	15	5	20
	O	13.00 (5.20)	15	5	20
4000 Hz	A	13.00 (7.10)	15	-5	20
	B	13.80 (6.00)	13.8	5	25
	AB	13.30 (5.20)	12.5	5	25
	O	12.80 (4.70)	15	0	20
8000 Hz	A	9.50 (8.60)	7.5	-5	25
	B	8.80 (6.30)	10	0	20
	AB	9.00 (7.00)	5	0	25
	O	9.30 (8.20)	10	-5	20
10 kHz	A	8.30 (9.90)	10	-10	20
	B	9.00 (9.30)	9	-5	25
	AB	8.50 (5.40)	10	0	15
	O	8.50 (8.80)	10	-10	20
12.5 kHz	A	12.50 (7.50)	12.5	0	25
	B	11.30 (5.60)	10	5	20
	AB	11.50 (7.60)	15	0	20
	O	11.00 (8.50)	10	-10	25
16 kHz	A	17.00 (8.80)	20	-5	25
	B	16.30 (4.30)	16.3	10	20
	AB	16.50 (5.20)	20	5	25
	O	16.30 (7.20)	20	0	25

Table 2. Mean, Median, Standard deviation, and range of tympanometry results across blood groups (n=20)

Tympanometry variables	Blood group	Mean (SD)	Median	Range	
				Minimum	Maximum
Compliance in ml	A	1.53 (0.23)	1.59	1.06	1.74
	B	1.54 (0.17)	1.55	1.20	1.94
	AB	1.51 (0.19)	1.62	1.05	1.74
	O	1.44 (0.21)	1.51	0.96	1.73
Ear canal volume in ml	A	1.25 (0.41)	1.22	0.62	1.91
	B	1.36 (0.38)	1.23	0.80	2.54
	AB	1.46 (0.44)	1.39	0.98	2.48
	O	1.72 (0.23)	1.54	1.11	3.92
Peak Pressure in daPa	A	-7.21 (7.62)	-4.50	-72.00	10.00
	B	-7.80 (8.45)	-4.00	-83.00	32.00
	AB	-7.30 (9.12)	-2.00	-36.00	74.00
	O	-2.35 (5.97)	-1.50	-29.00	10.00

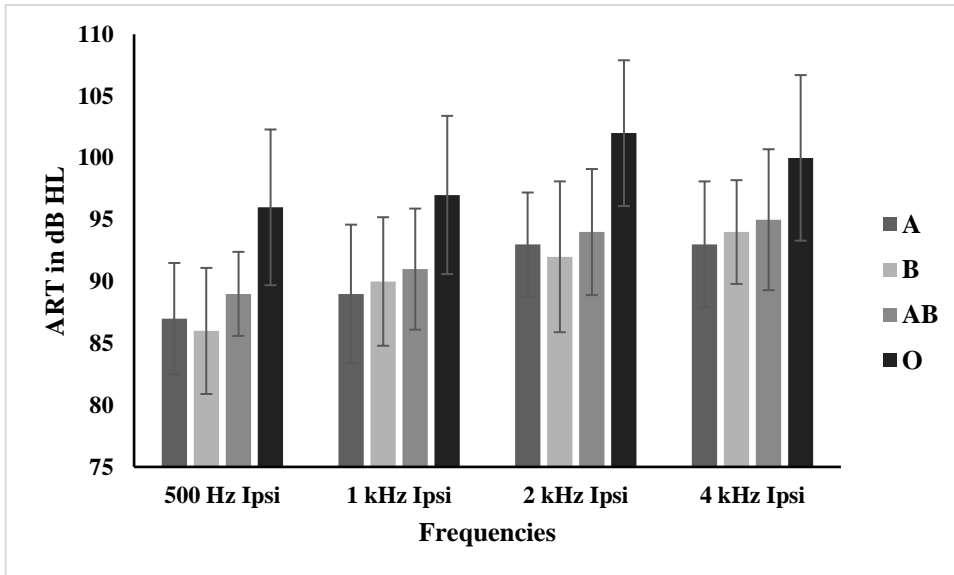


Figure 1. Mean and standard deviation of the ipsilateral acoustic reflex threshold for individuals with different blood groups. ART; acoustic reflex threshold

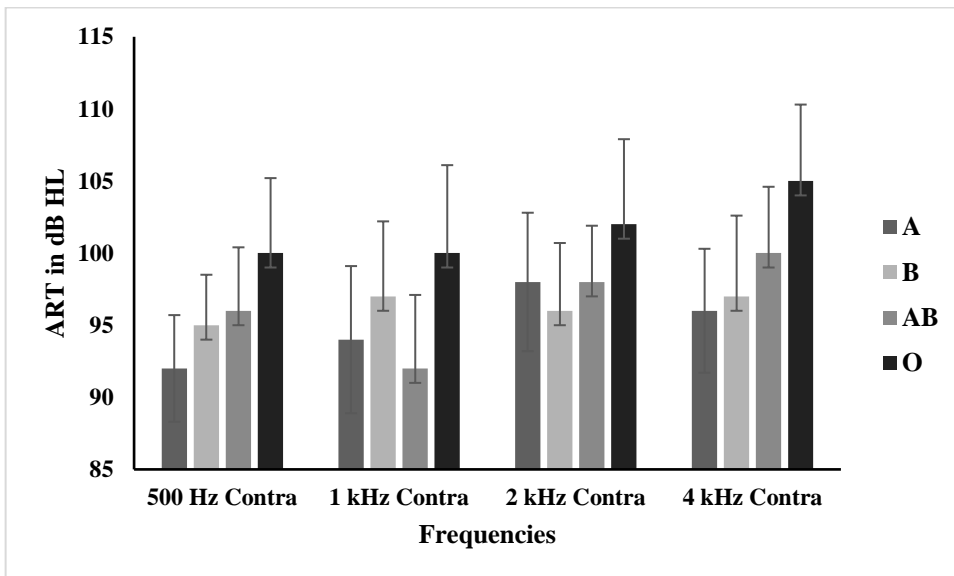


Figure 2. Mean and standard deviation of the contralateral acoustic reflex threshold for individuals with different blood groups. ART; acoustic reflex threshold