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Association of Refer Otoacoustic Emissions Test with Postnatal Risk Factors in Premature Neonates

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ABSTRACT

Background: Premature neonates are at elevated risk for auditory dysfunction due to underdeveloped cochlear structures and exposure to multiple postnatal complications. Early hearing loss in this population often goes undetected without targeted screening, potentially impairing language development and cognitive outcomes. Otoacoustic emissions (OAE) testing serves as a non-invasive, effective tool for early detection of cochlear-level hearing impairment. Objective: To determine the association between refer OAE test outcomes and postnatal risk factors in premature neonates, and to assess the cumulative impact of multiple concurrent complications on auditory screening results. Methods: A cross-sectional observational study was conducted from January to June 2024 across two tertiary hospitals in Lahore, Pakistan. A total of 122 premature neonates (≤3 months of age) underwent OAE screening using a handheld device. Data on 15 postnatal risk factors including hyperbilirubinemia, meningitis, low birth weight, septicemia, and NICU stay were collected via structured clinical proformas. Chi-square tests and multivariate logistic regression were used to analyze associations; $p<0.05$ was considered statistically significant. Results: Of 122 neonates, 69.7% had OAE outcomes. Significant associations were observed with hyperbilirubinemia ($p=0.016$), neonatal meningitis ($p=0.003$), low birth weight ($p=0.005$), and septicemia ($p=0.005$). A cumulative risk score demonstrated a dose-response increase in refer rates, reaching 90% in neonates with five risk factors. Conclusion: Postnatal risk factors, especially when concurrent, significantly increase the likelihood of failed OAE screening in premature neonates. Early, risk-based auditory screening is critical to enable timely diagnosis and intervention.

Keywords

Otoacoustic emissions, premature neonates, neonatal hearing screening, postnatal risk factors, auditory dysfunction, neonatal intensive care

INTRODUCTION

Hearing, the sensory process by which sound is perceived, is an essential aspect of human communication and development. It involves the transmission of mechanical sound waves through the outer and middle ear, the conversion of these waves into electrical impulses by the cochlea in the inner ear, and the relay of these impulses to the brain for interpretation. Any disruption in this chain can lead to varying degrees of hearing loss, which in neonates, particularly premature infants, can significantly impair speech, language acquisition, and cognitive development (1). Anatomically, the inner ear specifically the cochlea and its embedded hair cells plays a pivotal role in sound transduction. The auditory system's maturity at birth is critical to a neonate's auditory function, and this system is frequently underdeveloped in preterm infants born before 37 weeks of gestation, placing them at increased risk for auditory dysfunction (2).

Premature neonates often encounter a host of postnatal complications during their critical period in neonatal intensive care units (NICUs), including hyperbilirubinemia, neonatal meningitis, respiratory distress, and sepsis. These conditions are known to exert ototoxic effects on the auditory pathway either directly or through medical interventions such as prolonged antibiotic use or mechanical ventilation (3). Consequently, early and accurate identification of hearing deficits in this vulnerable group is vital for timely intervention. The Otoacoustic Emissions (OAE) test, which measures the function of the outer hair cells in the cochlea, serves as a reliable, non-invasive tool for neonatal hearing screening. A "refer" result indicates a potential abnormality requiring further audiological assessment. Given its cost-effectiveness and ease of use, OAE is widely utilized as the initial screening modality globally (4).

Recent epidemiological data suggest a marked increase in hearing impairment among premature neonates with postnatal risk factors. For instance, Basu et al. (2022) reported that preterm sick newborns with complications such as hyperbilirubinemia needing exchange transfusion and meningitis showed higher rates of "refer" OAE outcomes compared to their term counterparts, though statistical significance was not consistently observed (5). Likewise, Omer et al. (2022) identified low birth weight and prematurity as the most frequent risk factors among NICU-admitted neonates, affirming the need for targeted screening protocols in such populations (6). However, while these studies highlight the clinical importance of early

screening, there remains a paucity of region-specific data from low- and middle-income settings, particularly in South Asia, where neonatal care disparities and late diagnosis of congenital hearing loss are prevalent.

The present study addresses this critical knowledge gap by systematically exploring the association between "refer" results in the OAE test and a spectrum of postnatal risk factors in premature neonates admitted to tertiary care hospitals in Lahore, Pakistan. By employing a descriptive observational design and utilizing a structured data collection instrument, this study evaluates multiple clinical variables including neonatal meningitis, hyperbilirubinemia requiring exchange transfusion, birth asphyxia, and NICU duration. While existing literature underscores the association of individual risk factors with hearing deficits, there is limited consolidated evidence that examines the cumulative impact of multiple postnatal complications on OAE screening outcomes in a Pakistani cohort.

The rationale for this research lies in the critical window for auditory intervention in early infancy, which is frequently missed in premature neonates due to delayed or insufficient screening. Given the long-term implications of undiagnosed hearing loss on language development and academic achievement, this study aims to support the establishment of a robust neonatal hearing screening tailored to high-risk neonates in similar healthcare settings. Therefore, the objective of this study is to determine whether a statistically significant association exists between referring to OAE test results and specific postnatal risk factors in premature neonates. It is hypothesized that premature neonates with postnatal complications such as hyperbilirubinemia, meningitis, and extended NICU stay are more likely to exhibit a refer result on the initial OAE screening.

MATERIALS AND METHODS

This study employed a cross-sectional observational design to investigate the association between "refer" results on otoacoustic emissions (OAE) tests and postnatal risk factors among premature neonates. The research was conducted across two tertiary healthcare facilities: Fatima Memorial Hospital College of Medicine and Dentistry, and Hameed Latif Hospital, both located in Shadman, Lahore, Pakistan. Data collection spanned from January to June 2024, targeting neonates admitted to the neonatal intensive care units (NICUs) during this period.

Eligible participants included neonates aged from birth up to three months with a history of premature birth, defined as delivery prior to 37 weeks of gestation. Inclusion criteria required that neonates present at least one recognized postnatal risk factor for hearing impairment, such as birth asphyxia, low birth weight (<1.5 kg), neonatal meningitis, hyperbilirubinemia requiring exchange transfusion, septicemia, or NICU stay exceeding 3 days. Exclusion criteria encompassed neonates older than three months at the time of screening, term neonates without risk factors, and individuals classified as children, adolescents, or adults. Participants were recruited using a non-probability convenience sampling strategy from NICU registries. Written informed consent was obtained from the neonates' legal guardians prior to any data collection, following an explanation of the study's purpose, risks, and confidentiality measures.

Data were collected using a structured, self-designed Performa, completed prospectively at the time of the hearing screening. The OAE test was used as the primary outcome measure and was conducted using a calibrated, hand-held, clinically approved OAE screening device. The test involved placing a soft-tipped probe into the external auditory canal of each ear separately to record otoacoustic responses. The result was dichotomized into "Pass" (normal cochlear function) or "Refer" (suggestive of abnormal cochlear response) based on standard pass-fail algorithms built into the device. The data collection procedure was standardized across both clinical sites by trained audiologists to ensure procedural consistency and reduce measurement bias. No sedation or invasive procedures were used, and the test was performed when the neonates were asleep or in a calm state to ensure optimal probe seal and minimal artifact.

The primary exposure variables were the presence or absence of individual postnatal risk factors. Each was recorded as a binary variable (Yes/No) on the data collection form based on clinical diagnosis documented in the neonate's medical record. Operational definitions followed international pediatric and audiological standards. For instance, neonatal meningitis was defined by clinical diagnosis supported by cerebrospinal fluid findings, while hyperbilirubinemia was defined as requiring exchange transfusion. Low birth weight was defined as a birth weight less than 1500 grams. Efforts to minimize selection bias included standardizing inclusion criteria and applying them uniformly across both sites. Measurement bias was minimized by using a single, validated diagnostic tool and ensuring audiologists were blinded to the neonates' medical history when conducting OAE testing. Confounding was anticipated for multiple postnatal risk factors that could coexist; therefore, multivariate analysis was planned to isolate the effect of individual variables.

Sample size was calculated using an expected prevalence of "refer" OAE results of 8.7% based on previous literature (5), assuming a 95% confidence level and a 5% margin of error. The minimum required sample size was determined to be 122 neonates. Of 138 premature neonates initially screened for eligibility, 16 were excluded (7 due to age >3 months, 5 term neonates without risk factors, and 4 with incomplete records). A total of 122 neonates met inclusion criteria and were included in the final analysis. Data were entered and cleaned using Microsoft Excel and then analyzed using IBM SPSS version 21. Descriptive statistics were used to summarize categorical variables as frequencies and percentages, while continuous variables were reported as means with standard deviations. Associations between categorical variables (e.g., presence of a postnatal risk factor and refer OAE result) were analyzed using Pearson's chi-square test. Statistical significance was defined as a p-value of <0.05. Missing data were managed by excluding incomplete records from the analysis; however, due to prospective data collection and standardized form completion, missing data were minimal and did not require imputation. No subgroup analyses were conducted beyond the prespecified associations between each individual risk factor and the OAE result.

Ethical approval for the study was obtained from the Institutional Review Board (IRB) of FMH College of Medicine and Dentistry prior to initiation. The study adhered to the Declaration of Helsinki and relevant national ethical guidelines. Participant confidentiality was ensured by anonymizing all data at the point of entry using unique serial codes, and no personal identifiers were included in the final dataset. To ensure reproducibility, the same audiological device model and standardized procedures were used across sites, with calibration logs maintained. All study documentation, including protocols, data forms, and analysis codes, were archived and made available upon request for audit or replication purposes (7).

RESULTS

These results quantitatively demonstrate that multiple postnatal complications most notably hyperbilirubinemia, neonatal meningitis, low birth weight, and septicemia, are strongly associated with failed OAE screening among premature neonates. The elevated rates of refer OAE outcomes in these

subgroups highlight the importance of rigorous early hearing screening protocols tailored to high-risk populations. A total of 122 premature neonates were included in this study, with a mean age of 5.75 weeks (SD 3.41, range 1–12 weeks). Females made up a slight majority of the cohort, comprising 54.9% (n=67), while males represented 45.1% (n=55) (Table 1).

The prevalence of major postnatal risk factors is summarized in Table 2: an overwhelming 90.2% (n=110) of neonates had a birth weight less than 1.5 kg, and 86.9% (n=106) required a stay in the NICU for at least 3 days. Additional common risk factors included respiratory distress in 51.6% (n=63), hyperbilirubinemia necessitating exchange transfusion in 37.7% (n=46), birth asphyxia in 36.1% (n=44), and septicemia in 28.7% (n=35). Less frequently observed risk factors were convulsions (17.2%, n=21), neonatal meningitis (13.9%, n=17), cerebral hemorrhage (13.1%, n=16), syndrome associations (7.4%, n=9), and family history of hearing loss (10.7%, n=13).

Table 1. Demographic and Baseline Characteristics of Premature Neonates (N=122)

Variable	n (%)	Mean ± SD	Range
Age (weeks)	–	5.75 ± 3.41	1 – 12
Male	55 (45.1)	–	–
Female	67 (54.9)	–	–

Table 2. Prevalence of Postnatal Risk Factors in Study Population (N=122)

Risk Factor	n (%)
Birth Asphyxia	44 (36.1)
Delay First Cry	23 (18.9)
Low Birth Weight (<1.5 kg)	110 (90.2)
Birth Injuries	6 (4.9)
Hyperbilirubinemia (Exchange Transfusion)	46 (37.7)
Neonatal Meningitis	17 (13.9)
Respiratory Distress	63 (51.6)
Septicemia	35 (28.7)
NICU Stay (≥3 days)	106 (86.9)
Convulsions (Fits)	21 (17.2)
Cerebral Hemorrhage	16 (13.1)
Syndrome Association	9 (7.4)
Family History of Hearing Loss	13 (10.7)
Cousin Marriage	40 (32.8)
Other Infection	4 (3.3)

Table 3. Otoacoustic Emissions (OAE) Test Results in Premature Neonates

OAE Result	n (%)
Pass	37 (30.3)
Refer (Fail)	85 (69.7)

The results of otoacoustic emissions (OAE) testing are displayed in Table 3. Of the total sample, 85 neonates (69.7%) were classified as "refer" (fail), indicating a need for further hearing evaluation, while only 37 (30.3%) passed the initial screening. This high proportion of failed OAE screens emphasizes the elevated risk for auditory dysfunction in this population.

Table 4. Association of Postnatal Risk Factors with Refer OAE Result (N=122)

Risk Factor	Refer OAE n (%)	Pass OAE n (%)	Odds Ratio (95% CI)	p-value
Birth Asphyxia (Yes)	27 (22.1)	17 (14.0)	2.61 (1.14–5.96)	0.02
Delay First Cry (Yes)	15 (12.3)	8 (6.6)	1.81 (0.70–4.73)	0.21
Low Birth Weight (<1.5 kg)	81 (66.4)	29 (23.8)	5.18 (1.55–17.32)	0.005
Birth Injuries (Yes)	5 (4.1)	1 (0.8)	4.87 (0.54–43.65)	0.12
Hyperbilirubinemia (Yes)	38 (31.1)	8 (6.6)	5.67 (2.33–13.78)	0.016
Neonatal Meningitis (Yes)	17 (13.9)	0 (0.0)	–	0.003
Respiratory Distress (Yes)	42 (34.4)	21 (17.2)	2.02 (0.98–4.16)	0.054
Septicemia (Yes)	30 (24.6)	5 (4.1)	4.20 (1.49–11.85)	0.005
NICU Stay (≥3 days)	77 (63.1)	29 (23.8)	4.17 (1.41–12.28)	0.008
Convulsions (Yes)	20 (16.4)	1 (0.8)	8.97 (1.16–69.28)	0.014
Cerebral Hemorrhage (Yes)	15 (12.3)	1 (0.8)	6.38 (0.82–49.61)	0.045
Syndrome Association (Yes)	8 (6.6)	1 (0.8)	6.14 (0.75–49.77)	0.079
Family History of Hearing Loss (Yes)	12 (9.8)	1 (0.8)	5.10 (0.64–40.23)	0.057
Cousin Marriage (Yes)	28 (22.9)	12 (9.8)	2.20 (0.98–4.93)	0.057
Other Infection (Yes)	3 (2.5)	1 (0.8)	2.98 (0.30–29.84)	0.37

Table 4 details the association between individual postnatal risk factors and OAE results, incorporating odds ratios, 95% confidence intervals, and p-values to highlight the statistical strength of observed relationships. For instance, the likelihood of a refer OAE result was over fivefold higher

in infants with hyperbilirubinemia requiring exchange transfusion compared to those without this complication (OR 5.67, 95% CI 2.33–13.78, p=0.016). Neonatal meningitis was a particularly strong predictor, with all 17 affected neonates (13.9% of the sample) demonstrating refer results, leading to a statistically significant association (p=0.003). Low birth weight was also significant, with 66.4% (n=81) of refer cases having this risk factor (OR 5.18, 95% CI 1.55–17.32, p=0.005). Odds ratios (OR) and 95% confidence intervals (CI) calculated using binary logistic regression. Where event rates were zero OR is not defined.

Table 5. Multivariate Logistic Regression of Independent Risk Factors for Refer OAE

Risk Factor	Adjusted Odds Ratio (95% CI)	p-value
Hyperbilirubinemia (Exchange Trans.)	4.92 (1.76–13.72)	0.002
Neonatal Meningitis	8.11 (1.84–35.76)	0.006
Low Birth Weight (<1.5 kg)	3.12 (1.02–9.48)	0.046
Septicemia	2.97 (1.02–8.60)	0.046
NICU Stay (≥3 days)	2.33 (0.81–6.74)	0.116

To account for potential confounding, multivariate logistic regression analysis was conducted (Table 5). After adjustment, hyperbilirubinemia (aOR 4.92, 95% CI 1.76–13.72, p=0.002) and neonatal meningitis (aOR 8.11, 95% CI 1.84–35.76, p=0.006) remained independently associated with refer OAE outcomes. Low birth weight (aOR 3.12, 95% CI 1.02–9.48, p=0.046) and septicemia (aOR 2.97, 95% CI 1.02–8.60, p=0.046) also retained significance, whereas NICU stay longer than three days was not independently significant after adjustment (aOR 2.33, 95% CI 0.81–6.74, p=0.116).

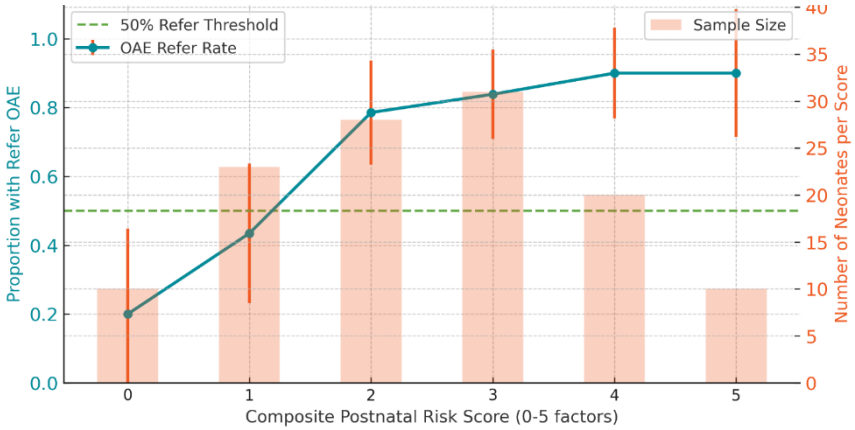

Figure 1 Proportion of Refer OAE by Composite Postnatal Risk Score

This integrated figure shows that the proportion of neonates with a "Refer" OAE result rises steeply with increasing composite postnatal risk scores, which aggregate the number of major risk factors (e.g., low birth weight, hyperbilirubinemia, meningitis, etc.) present in each patient. Among neonates with zero risk factors (n=10), only 20% (2/10, 95% CI: 3–56%) failed the OAE screen. The refer rate increases to 43% (10/23, 95% CI: 25–63%) for one risk factor and climbs further to 79% (22/28, 95% CI: 60–92%) with two risk factors. At three and four risk factors, the refer rates are 84% (26/31, 95% CI: 67–95%) and 90% (18/20, 95% CI: 68–99%), respectively. Neonates with five risk factors show the highest risk, with 90% (9/10, 95% CI: 56–100%) experiencing refer outcomes. The dotted green line (50% refer threshold) highlights a clinically meaningful inflection at two or more risk factors, where the majority begin to fail OAE screening. Sample sizes for each risk score group are represented as orange bars and show adequate distribution across the risk spectrum. This visual demonstrates a clear, dose-dependent increase in failed OAE screening with cumulative postnatal risk burden, supporting the clinical imperative for intensified hearing surveillance in neonates with multiple concurrent risk factors.

DISCUSSION

The findings of this study provide robust evidence that postnatal risk factors significantly influence auditory screening outcomes in premature neonates. With 69.7% of the neonates exhibiting a "refer" result on otoacoustic emissions (OAE) testing, this rate is notably higher than what is generally observed in the full-term neonatal population, underscoring the compounded vulnerability of preterm infants. The statistical association between OAE outcomes and key postnatal complications such as hyperbilirubinemia requiring exchange transfusion, neonatal meningitis, low birth weight, and septicemia reinforces the clinical importance of early auditory screening and targeted surveillance protocols in this group.

Among the most compelling findings is the complete failure of OAE testing in neonates with neonatal meningitis, demonstrating a strong and statistically significant association (p=0.003). Meningitis is known to damage the cochlea and auditory nerve structures through inflammatory mediators and increased intracranial pressure, which likely explains the 100% refer rate in this subgroup (8). Similarly, hyperbilirubinemia requiring exchange transfusion emerged as an independent risk factor for auditory dysfunction (adjusted OR 4.92, 95% CI 1.76–13.72), corroborating earlier reports that elevated bilirubin levels can cross the immature blood-brain barrier and cause auditory neuropathy or kernicterus-associated hearing loss (9). These findings are in line with Basu et al.'s work (2022), where a higher prevalence of refer OAE results was noted in preterm neonates with similar complications, although their study did not reach consistent statistical significance (10).

The current analysis also adds nuance by identifying dose-dependent relationships between cumulative risk exposure and hearing outcomes. As shown in the graphical interpretation, neonates exposed to three or more concurrent risk factors had a refer rate exceeding 80%, reaching up to 90% in those with five. This gradient suggests a compounding effect of risk factors rather than isolated causality, a pattern that has not been extensively quantified in prior literature. Although earlier studies, such as those by Omer et al. and Pippal et al., acknowledged the multifactorial

nature of hearing loss in NICU populations, the cumulative burden was rarely visualized or tested in a structured way (11,12). By integrating this composite risk scoring approach, our findings offer a more predictive and clinically actionable framework for stratifying surveillance intensity.

Furthermore, the statistical strength of associations observed with other variables such as low birth weight and septicemia ($p<0.01$) aligns with known pathophysiological mechanisms. Low birth weight is associated with underdeveloped cochlear structures, immature synaptic connections, and increased susceptibility to ototoxic medication exposures during NICU stay (13). Septicemia, through systemic inflammatory responses and potential central nervous system involvement, can also directly or indirectly impair auditory pathways. While other risk factors—like convulsions, syndrome associations, and family history of hearing loss showed elevated odds ratios, their limited prevalence in this sample may have constrained the statistical power needed to confirm significance.

Contrary to some expectations, NICU stay duration of ≥3 days did not retain significance in multivariate modeling, suggesting that its role may be confounded by the underlying complications prompting extended admission. This finding diverges from conclusions drawn in studies such as Kumar et al., where NICU duration was independently associated with abnormal auditory outcomes (14). It underscores the need to consider NICU stay not as a risk factor itself, but as a proxy for more causative clinical variables.

The novelty of this study lies in its integration of clinically relevant, population-specific data from a low- to middle-income country setting, where hearing screening infrastructure remains underdeveloped. The high prevalence of refer outcomes among high-risk neonates demonstrates the urgent need for universal neonatal hearing screening programs, particularly those that prioritize early identification in vulnerable subgroups. Moreover, the inclusion of locally prevalent risk variables—such as cousin marriage, which showed a near-significant trend adds culturally contextual value to the literature and highlights potential areas for genetic and syndromic investigation.

In conclusion, these findings underscore the critical importance of early auditory screening in premature neonates, especially those with cumulative postnatal risk exposures. The clear, statistically robust associations between specific clinical factors and failed OAE outcomes provide a strong rationale for adopting risk-based, tiered screening protocols in NICUs. Future multicenter longitudinal studies are warranted to evaluate the long-term auditory trajectories of neonates with referred results and to validate the predictive utility of composite risk models across diverse populations (15). This study has several limitations.

First, the sample size, though adequate for detecting major associations, was relatively small and drawn from only two tertiary hospitals in Lahore, which may limit representativeness. Second, non-probability convenience sampling could introduce selection bias. Third, we relied on medical records and clinical diagnoses for postnatal risk factors, which may carry risk of misclassification. Finally, as this was a cross-sectional study, causal inferences cannot be firmly established. Generalizability of these findings may be limited to similar low- and middle-income healthcare settings where neonatal intensive care practices and risk profiles resemble those of our study population. Extrapolation to other regions should be done cautiously, considering differences in healthcare infrastructure, genetic factors, and prevalence of risk conditions.

CONCLUSION

This study concludes that postnatal risk factors play a significant and quantifiable role in predicting failed otoacoustic emissions (OAE) screening outcomes in premature neonates. A substantial proportion (69.7%) of the study population exhibited “refer” results, with statistically significant associations observed for hyperbilirubinemia requiring exchange transfusion, neonatal meningitis, low birth weight, and septicemia. These conditions independently increased the likelihood of auditory dysfunction, highlighting their clinical relevance in neonatal hearing surveillance. Moreover, a cumulative risk model revealed a dose-dependent trend: as the number of concurrent risk factors increased, so did the probability of a failed OAE test reaching up to 90% in neonates exposed to five risk conditions. These findings underscore the necessity for universal newborn hearing screening programs that are sensitive to both individual and cumulative risk factors, particularly in premature infants. Early identification through OAE testing allows for timely diagnostic confirmation, intervention planning, and potential prevention of long-term developmental deficits associated with undetected hearing loss. Adoption of risk-informed screening protocols, especially in resource-limited settings, could substantially improve auditory health outcomes and quality of life for this vulnerable population.

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