Statistical analysis of the incidence of urinary tract infection in neonates with significant indirect Hyperbilirubinemia

Sruthi Srikantan

Email: sruthi.srikantan@uconn.edu
NetId: srs18007

Introduction

Hyperbilirubinemia, characterized by elevated bilirubin levels, often manifests as jaundice in newborns and has been increasingly linked to urinary tract infections (UTIs)(Baz et al 2021). Neonatal jaundice is a common reason for admission and is observed in the first week of life for 60% of full term infants and 80% in premature infants. Current institutions such as the American Academy of Pediatrics do not recommend the use of urinalysis to investigate the presence of UTIs in neonatal jaundice(Ozcan 2017). This paper presents findings from a case-control study involving 100 jaundiced infants and 50 matched controls, aimed at exploring the association between hyperbilirubinemia and UTIs. Gestational age for all of the subjects was greater than 35 weeks. The study focused on assessing UTI prevalence through the detection of pyuria and bacterial culture. By investigating this relationship, the study aims to contribute to early UTI recognition in jaundiced neonates, potentially improving clinical outcomes and guiding appropriate management practices.

Description of Data

In this study, the researchers divided 150 neonates into two groups, with 100 in the treatment and 50 in the control. The treatment group consists of neonates with positive cases of jaundice and the control group consists of jaundice negative individuals. 43 of the neonates in the control group were male and 57 were female. 25 of the neonates in the control group were female and the other 25 in the control group were male. All neonates in the study were greater than gestational age of 35 weeks. Urine samples were collected by catheterization from neonates and a urine analysis was conducted. The samples were then examined microscopically under a high power field(HPF) for pyuria. Samples with pyuria or greater than 5 white blood cell(WBC) count per HPF confirmed the presence of a UTI. Additionally, a culture was done to examine the bacteria present in each positive UTI case. The paper states that the infants were matched based

on specific criteria to ensure comparability between the case group (treatment) and the control group. However, the paper does not directly state the exact factors on which the infants were matched such as sex, gestational age, etc. This lack of clarity does make it difficult to assess whether this research is a true matched case control study. Since this information remains ambiguous I will assume the data is independent and unmatched. Table 1 of the paper looks at the mean and standard deviation of different variables in the treatment and control group. These variables include Age on admission, Weight(kg), Total bilirubin(mg/dl), direct bilirubin(mg/dl), Hemoglobin(gm/dl), Reticulocyte count(%), Urea(mg/dl), and Creatinine(mg/dl). Based on the mean and standard deviations of both groups, the values appear close in range between the treatment and control groups regarding the means and standard deviations. The mean and standard deviations were also calculated in the R output. Table 2 of the paper calculates the counts and percentages of the bacteria present in the positive case of UTI. The sample size for the positive cases for UTI in the treatment group was 11. From this table we see that E. coli makes up 36.4% of the bacteria found in the urine samples. Table 3 exclusively looks at the treatment group and splits the treatment into two groups, group A and group B. Group A is made up of the neonates with positive cases of UTI(n=11) and group B are the negative cases of UTI(n=89). The first section of Table 3 looks at the mean and standard deviation of different variables in group A and group B. These variables include Age on admission, Weight(kg), Total bilirubin(mg/dl), direct bilirubin(mg/dl), Hemoglobin(gm/dl), Reticulocyte count(%), Urea(mg/dl), and Creatinine(mg/dl). Again the is very little difference between groups A and B regarding each of the variables' mean and standard deviation. The second section of table 3 looked at the counts and percentages of the sexes between group A and group B. The splits between the sexes were relatively equal, with the female sex making up a slightly greater

percentage in both groups. The third section of Table 3 looks at the maternal history for both groups. Here 3 variables linked to maternal infection were identified. These variables include Negative, UTI, and PROM. Additionally, the counts and percentages were calculated for each of the 3 variables. Interestingly, UTI makes up the highest percentage of Group A at 45.4% and Negative makes up 86.5% of group B. This could indicate that a UTI infection could be passed on from the mother to the infant. The last section of Table 3 looks at Maternal history of chronic disease. This section identifies 3 variables, Non, Diabetes, and Hypertension, for groups A and B. Non makes up the highest percentage for group A at 72.7% and the highest for group B at 69.7%.

Methods

This paper uses the Chi-square test to analyze the significance of a variety of variables and the p value is examined. Assumptions of the chi-square test are that all observations are independent, both variables are categorical, expected frequencies should be 5 or greater for at least 80% of the cells, and random sampling of the participants to make sure an individual does not belong to more than 1 cell. The formula for the chi-square test is seen below:

$$\chi^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

Where:

O indicates the observed cases

E indicates the expected value

χ 2 is the Chi-square value

Assumptions made by the researchers include the neonates in this study were representative of the broader population of neonates with significant unexplained indirect hyperbilirubinemia and those without hyperbilirubinemia. The research is unmatched and the sample size was adequate with each cell in the contingency table greater than or equal to 5. The variables observed in the chi square test were Age on admission, Weight(kg), Total bilirubin(mg/dl), direct bilirubin(mg/dl), Hemoglobin(gm/dl), Reticulocyte count(%), Urea(mg/dl), and Creatinine(mg/dl) against treatment and control for table 1. For table 3 we compare these same sets of variables against groups A and B. These variables are categorical and meet the assumptions for the chi square test. Additionally, the researchers assumed a significance level equal to 0.05 when observing p values. The null hypothesis(H0) for the chi square test states that there is no statistical difference between the variables on treatment and control groups for table 1. The alternative hypothesis(HA) states there is a statistical difference between both control and treatment. For table 3 the null hypothesis for the chi square test states there is no statistical difference between the variables on group A and group B. The alternative hypothesis(HA) states there is a statistical difference between both groups. The paper looks at the p value for each of the variables in tables 1 and 3. In table 3 the researchers also conducted a Fisher's exact test for the sex of the neonates of groups A and B. The researchers also obtained a p value through the Monte Carlo test for maternal infection of groups A and B and maternal history of chronic disease. However, since this test was not covered in class I have used Fisher's exact test cases when r>2 or c>2 for the rxc table. In contingency tables with more than two rows or columns, the Fisher's exact test is useful when the sample size is small and the expected cell counts are less than 5, making the traditional chi-square test less reliable. It provides an exact p-value,

making no assumptions about the distribution of the data. The equation for the Fisher's exact test is:

$$p_{n_{11}} = \frac{\binom{n_{1.}}{n_{11}}\binom{n_{2.}}{n_{.1}-n_{11}}}{\binom{n}{n_{.1}}}.$$

Where:

n1,n2,n11,n.1 indicate the values of the contingency table

p is the p value

n is the total frequency

The assumptions of Fisher's exact test are that all margins of the contingency table are fixed, all observations are independent, there is a small sample size where the frequencies of the table are less than 5, and the data is categorical. This study meets these assumptions as all participants are independent, there is a small sample size of 100, and the data for sex, maternal infection, and maternal history is categorical. Additionally, the researchers note the significance level is equal to 0.05.

Analysis of Data

Baz et al. used the IBM SPSS in their analysis for the chi-squared and the Fisher's exact test, however, I used R to replicate the results. Looking at table 1, we see the researchers used a chi-square test to analyze the p value for the variables Age on admission, Weight(kg), Total bilirubin(mg/dl), Direct bilirubin(mg/dl), Hemoglobin(gm/dl), Reticulocyte count(%), Urea(mg/dl), and Creatinine(mg/dl) for the treatment and control groups. Based on a significance

level of 0.05, Total bilirubin(mg/dl) and Direct bilirubin(mg/dl) have a p value less than 0.001 which is statistically significant since it is less than significance level. To replicate these results in R, I used the chisq.test() function to calculate the p values. From my analysis I also came to the same conclusion that Total bilirubin(mg/dl) and Direct bilirubin(mg/dl) were statistically significant. Total bilirubin(mg/dl) had a p value of 615e-08 and Direct bilirubin(mg/dl) had a p value of 0.03734. Since the p values are less than 0.05, these variables are significant which means that there is an association between. Therefore, for Total bilirubin and Direct bilirubin we reject the null and these two variables differ in their levels between treatment and control. In table 3, the researchers also used a chi-square test to analyze the p value for the variables Age on admission, Weight(kg), Total bilirubin(mg/dl), Direct bilirubin(mg/dl), Hemoglobin(gm/dl), Reticulocyte count(%), Urea(mg/dl), and Creatinine(mg/dl) for group A and group B. At a significance level of 0.05, none of the variables appear to be statistically significant. I replicated these results by using the chisq.test() function in R. From my analysis I found that my results varied in that Total bilirubin(mg/dl), Hemoglobin(gm/dl),Reticulocyte count(%), and Urea(mg/dl) were all statistically significant. Total bilirubin(mg/dl) had a p value of 0.002, Hemoglobin(gm/dl) has a value of 0.0004, and Reticulocyte count(%) and Urea(mg/dl) had a value of 0.003. In the second section of table 3, the researchers used a fisher's exact test to observe the significance of sex on groups A and B. The results from this test also indicate that sex is not statistically significant. I used the fisher.test() function to calculate the p value between sex and UTI. The result from this replication is exactly the same as the paper where the p value is equal to 1. Therefore, we fail to reject the null and state sex is not statistically different between groups A and B. The third section of table 3 conducts a Monte Carlo test for maternal infection on groups A and B. The results show that maternal infection has a p value of 0.001 which is

statistically significant. To replicate these findinging I again used the fisher.test() function in R. From my results I was able to calculate a p value of 0.0007 which is close to the paper's p value. Therefore, we can reject the null and determine that Maternal infection is statistically different between groups A and B. The last section of table 3 conducts a Monte Carlo test on Maternal history of chronic disease on groups A and B. The results indicate a p value equal to 0.001 which is statistically significant. However, I could not conduct this test in R as the researchers did not provide the data for the treatment group.

Discussion

In reviewing the results obtained from the replication of Baz et al.'s study using R, several discrepancies emerged, particularly in the analysis of variables between treatment and control groups, as well as between groups A and B. These inconsistencies prompt a critical examination of the assumptions made in Section 3 of the paper and of the conclusions drawn. The first notable inconsistency arises from the analysis of Total bilirubin(mg/dl) and Direct bilirubin(mg/dl) between treatment and control groups. While Baz et al. reported statistically significant findings for both variables as both had a p value less than 0.001, replicating the analysis in R yielded consistent significance for Total bilirubin(mg/dl) with a p value of 6.15e-08 but only marginal significance for Direct bilirubin(mg/dl) with a p value of 0.037. Similarly, discrepancies in the analysis of variables between groups A and B cast doubt on the consistency of the results. While Baz et al. found no statistically significant associations for any variable, the replication using R revealed significant associations for Total bilirubin(mg/dl), Hemoglobin(gm/dl), Reticulocyte count(%), and Urea(mg/dl). This discrepancy may stem from variations in the statistical methods employed or potential differences in data preprocessing. For example, the article assumes matched paired between the neonates, however, due to a lack of

information on how the neonates were matched I assumed the neonates were unmatched. If information was provided on how the neonates were matched I could have used McNemar's chi square test as opposed to Pearson's chi-squared test. Additionally, the researchers never stated which chi-squared test was used, meaning I had to assume the Pearson's test was used. In contrast, the replication of Fisher's exact test for the association between sex and UTI yielded consistent results with the original study, reaffirming the non-significant relationship between these variables. This consistency lends support to the validity of the findings regarding sex differences between groups A and B. Additionally, maternal infection had similar results between the original paper and replicated findings, however, the paper did use a Monte Carlo test which causes the p values to vary slightly. Another cause of concern between the original paper and the replication was the lack of data provided by the researchers on maternal history of chronic illness, therefore it is challenging to validate the reported findings fully. Reflecting on the assumptions listed in Section 3 of the paper, it is evident that some of these assumptions may not have been fully justified. For example, when analyzing variables Total bilirubin(mg/dl), Hemoglobin(gm/dl), etc. against the treatment/control groups and group A/group B we see that these variables are not exactly categorical. The paper never mentions how the researchers created their contingency tables which made the process of recreating the tables difficult. Additionally, the researchers make the assumption that the neonates are matched and conduct the analysis of the data to support this assumption, however, due to a lack of information I could not meet this assumption in the paper. In conclusion, the replication of the results conclude that there are some statistical differences between the treatment/control groups and group A/group B that are worth exploring such as maternal infection and Total bilirubin(mg/dl) levels. Overall, further research

needs to be on this topic to support the connection between UTI and hyperbilirubinemia in neonates.

Works Cited

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