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Biostatistics for Research Beginners

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KEYWORDS :

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ABSTRACT

Biostatistics is the application of statistics to a wide range of topics in biology. Statistics is basically a way of thinking about data that are variable. Biostatistics encompasses the design of biological experiments, especially in medicine, pharmacy, agriculture and fishery; the collection summarization, and analysis of data from those experiments; and the interpretation of, and inference from, the results. More students are entering college having completed a statistics class, and more students are studying statistics at the research level. There is growing demand for a variety of strong research level programs in statistics to help prepare the next generation of students to make sense of the information around them. This review article deals with basic biostatistical concepts and their application to enable postgraduate medical and allied science students to analyze and interpret their study data and to critically interpret published literature.

Introduction

Statistics is an increasingly important discipline, spurred by the proliferation of complex and rich data as well as the growing recognition of the role that statistical analysis plays. in making evidence-based decisions. Statistics is basically a way of thinking about data that are variable. This article deals with basic biostatistical concepts and their application to enable postgraduate medical and research beginners to analyze and interpret their study data and to critically interpret published literature. Acquiring such skills currently forms an integral part of their research training. Self-motivation for effective learning and application of statistics is lacking. Statistics implies both, data and statistical methods. It can be considered as an art as well as science. Statistics can neither prove not disprove anything. It is just a tool. Statistics without scientific application has no roots. Thus, statistics may be defined as the discipline concerned with the treatment of numerical data derived from group of individuals. These individuals may be human beings, animals, or other organisms. Data are produced in the experiments using individuals. Data can be highly variable due to several factors, including genetics, age, sex, race, economic background, measurement techniques, and many others.

Biostatistics is a branch of statistics applied to biological or medical sciences. Biostatistics covers applications and contributions not only from health, medicines and, nutrition but also from fields such as genetics, biology, epidemiology, and many others[1]. Biostatistics mainly consists of various steps like generation of hypothesis, collection of data, and application of statistical analysis. To begin with, readers should know about the data obtained during the experiment, its distribution, and its analysis to draw a valid conclusion from the experiment. The appropriate statistical analysis depends on the research questions of interest, the study design and the nature of the variables being examined.

Statistical method has two branches one descriptive and other inferential. Descriptive statistics explain the distribution of population measurements by collecting types of data, estimates of central tendency (mean, mode and median), and measures of variability (standard deviation, correlation coefficient), whereas inferential statistics is used to express the level of certainty about estimates and includes hypothesis testing, standard error of mean, and confidence interval. We begin by discussing principles that informed our thinking. Then we consider skills that students should develop in their courses along with a summary of key curriculum topics.

Types of data

Observations recorded during research constitute data. There are three types of data i.e. nominal, ordinal, and interval data. Statistical methods for analysis mainly depend on type of data. Generally, data exhibit picture of the variability and central tendency. Therefore, it is most important to understand the types of data.

Nominal data: Where data is simply assigned "names" or categories based on the presence or absence of certain attributes/ characteristics without any ranking between the categories [2]. It also includes binominal data, which refers to two possible outcomes

2) Ordinal data: It is also called as ordered, categorical, or graded data. This type of data is expressed as scores or ranks. There is a natural order among categories, and they can be ranked or arranged in order [2]. To indicate the intensity of pain, it may also be expressed as scores (mild = 1, moderate = 2, severe = 3). Hence, data can be arranged in an order and rank.

Interval data: This type of data is characterized by an equal and definite interval between two measurements. For example, weight is expressed as 20, 21, 22, 23, 24 kg. The interval between 20 and 21 is same as that between 23 and 24. Interval type of data can be either continuous or discrete. A continuous variable can take any value within a given range..

Sometimes, certain data may be changed from one form to another form to decrease skewness and make it to follow the normal distribution. For example, drug doses are converted to their log values and plotted in dose response curve to obtain a straight line so that analysis becomes easy [3]. Data can be transformed by taking the logarithm, square root, or reciprocal. Logarithmic conversion is the most common data transformation used in medical research.

Measurement of central tendency

Measure of central tendency is a central value or a typical value for a probability distribution. It is occasionally called an average or just the center of the distribution. The most common measures of central tendency are the arithmetic mean, the median and the mode. A central tendency can be calculated for either a finite set of values or for a theoretical distribution, such as the normal distribution. Mean is the common measure of central tendency, most widely used in calculations of averages. It is least affected by sampling fluctuations. The mean of a number of individual values (X) is always nearer the true value of the individual value itself. It is calculated by adding up the individual values (Σx) and dividing the sum by number of items (n). Suppose height of 7 boys's is 60, 70, 80, 90, 90, 100, and 110 cms. Addition of height of 7 boys is 600 cm, so mean(X) = Σx/ n=600/7=85.71.

Median equals the observation in the center when all observations are ordered from smallest to largest; when there is an even number of observations the median is defined as the average of

the middle two values. It is used for scores and ranks. Median is a better indicator of central value when one or more of the lowest or the highest observations are wide apart or are not evenly distributed. Median in case of even number of observations is taken arbitrary as an average of two middle values, and in case of odd number, the central value forms the median. In above example, median would be 90. Mode is the most frequent value, or it is the point of maximum concentration. Most fashionable number, which occurred repeatedly, contributes mode in a distribution of quantitative data. In above example, mode is 90. Mode is used when the values are widely varying and is rarely used in medical studies. For skewed distribution or samples where there is wide variation, mode, and median are useful.

Even after calculating the mean, it is necessary to have some index of variability among the data. Range or the lowest and the highest values can be given, but this is not very useful if one of these extreme values is far off from the rest. At the same time, it does not tell how the observations are scattered around the mean. Therefore, following indices of variability play a key role in biostatistics.

Standard Deviation

Standard deviation (SD) indicates that the data points tend to be very close to the mean (also called expected value); a high standard deviation indicates that the data points are spread out over a large range of values.

SD describes the variability of the observation about the mean [4]. A useful property of the standard deviation is that, unlike the variance, it is expressed in the same units as the data. Note, however, that for measurements with percentage as the unit, the standard deviation will have percentage points as the unit. To describe the scatter of the population, most useful measure of variability is SD. Summary measures of variability of individuals (mean, median, and mode) are further needed to be tested for reliability of statistics based on samples from population variability of individual.

To calculate the SD, we need its square called variance. Variance is the average square deviation around the mean and is calculated by Variance = Σ(x-x-) 2/n OR Σ(x-x-) 2/n-1, now SD = √variance. SD helps us to predict how far the given value is away from the mean, and therefore, we can predict the coverage of values. SD is more appropriate only if data are normally distributed. If individual observations are clustered around sample mean (M) and are scattered evenly around it, the SD helps to calculate a range that will include a given percentage of observation. For example, if N ≥ 30, the range M ± 2(SD) will include 95% of observation and the range M ± 3(SD) will include 99% of observation. If observations are widely dispersed, central values are less representative of data, hence variance is taken. While reporting mean and SD, better way of representation is 'mean (SD)' rather than 'mean ± SD' to minimize confusion with confidence interval [5,6].

Correlation Coefficient

Correlation coefficient, also known as r, R, or Pearson's r, a measure of the strength and direction of the linear relationship between two variables that is defined as the (sample) covariance of the variables divided by the product of their (sample) standard deviations[7]. Correlation may be due to some direct relationship between two variables. This also may be due to some inherent factors common to both variables. The correlation is expressed in terms of coefficient. The correlation coefficient values are always between -1 and +1. If the variables are not correlated, then correlation coefficient is zero. The maximum value of 1 is obtained if there is a straight line in scatter plot and considered as perfect positive correlation. The association is positive if the values of x-axis and y-axis tend to be high or low together. On the contrary, the association is negative i.e. -1 if the high y axis values tends to go with low values of x axis and considered as perfect negative correlation. Larger the correlation coefficient, stronger is the association. A weak correlation may be statistically significant if the numbers of observation are large. Correlation between the two variables does not necessarily suggest the cause and effect relationship. It indicates the strength of association for any data in comparable terms as for example, correlation between height and weight, age and height, weight loss and poverty, parity and birth weight, socioeconomic status and hemoglobin. While performing these tests, it requires x and y variables to be normally distributed. It is generally used to form hypothesis and to suggest areas of future research.

Types of Distribution

Though this universe is full of uncertainty and variability, a large set of experimental/biological observations always tend towards a normal distribution. This unique behavior of data is the key to entire inferential statistics. There are two types of distribution.

1- Gaussian /normal distribution

If data is symmetrically distributed on both sides of mean and form a bell-shaped curve in frequency distribution plot, the distribution of data is called normal or Gaussian. The noted statistician professor Gauss developed this, and therefore, it was named after him. The normal curve describes the ideal distribution of continuous values i.e. heart rate, blood sugar level and Hb % level. In an ideal Gaussian distribution, the values lying between the points 1 SD below and 1 SD above the mean value (i.e. ± 1 SD) will include 68.27% of all values. The range, mean ± 2 SD includes approximately 95% of values distributed about this mean, excluding 2.5% above and 2.5% below the range. In ideal distribution of the values; the mean, mode, and median are

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equal within population under study [8]. Even if distribution in original population is far from normal, the distribution of sample averages tend to become normal as size of sample increases. This is the single most important reason for the curve of normal distribution. Various methods of analysis are available to make assumptions about normality, including't' test and analysis of variance (ANOVA). In normal distribution, skew is zero. If the difference (mean–median) is positive, the curve is positively skewed and if it is (mean–median) negative, the curve is negatively skewed, and therefore, measure of central tendency differs.

2- Non-Gaussian (non-normal) distribution

If the data is skewed on one side, then the distribution is non-normal. It may be binominal distribution or Poisson distribution. In binominal distribution, event can have only one of two possible outcomes such as yes/no, positive/negative, survival/death, and smokers/non-smokers. When distribution of data is non-Gaussian, different test like Wilcoxon, Mann-Whitney, Kruskal-Wallis, and Friedman test can be applied depending on nature of data.

Standard Error of Mean

Since we study some points or events (sample) to draw conclusions about all patients or population and use the sample mean (M) as an estimate of the population mean (M^1), we need to know how far M can vary from M^1 if repeated samples of size N are taken. A measure of this variability is provided by Standard error of mean (SEM), which is calculated as ($SEM = SD/\sqrt{n}$). SEM is always less than SD. What SD is to the sample, the SEM is to the population mean.

Confidence Interval (CI) OR (Fiducial limits)

Confidence limits are two extremes of a measurement within which 95% observations would lie. These describe the limits within which 95% of the mean values if determined in similar experiments are likely to fall. The value of 't' corresponding to a probability of 0.05 for the appropriate degree of freedom is read from the table of distribution. By multiplying this value with the standard error, the 95% confidence limits for the mean are obtained as per formula below.

Lower confidence limit = mean - ($t_{0.05} \times SEM$)

Upper confidence limit = mean + ($t_{0.05} \times SEM$)

If $n > 30$, the interval $M \pm 2(SEM)$ will include M with a probability of 95% and the interval $M \pm 2.8(SEM)$ will include M with probability of 99%. These intervals are, therefore, called the 95% and 99% confidence intervals, respectively [9]. The important difference between the 'p' value and confidence interval is that confidence interval represents clinical significance, whereas 'p' value indicates statistical significance. Therefore, in many clinical studies, confidence interval is preferred instead of 'p' value,[4] and some journals specifically ask for these values.

Various medical journals use mean and SEM to describe variability within the sample. The SEM is a measure of precision for estimated population mean, whereas SD is a measure of data variability around mean of a sample of population. Hence, SEM is not a descriptive statistics and should not be used as such [10]. Correct use of SEM would be only to indicate precision of estimated mean of population.

Null Hypothesis

The primary object of statistical analysis is to find out whether the effect produced by a compound under study is genuine and is not due to chance. Hence, the analysis usually attaches a test of statistical significance. First step in such a test is to state the null hypothesis. In null hypothesis (statistical hypothesis), we make assumption that there exist no differences between the two groups. Alternative hypothesis (research hypothesis) states that there is a difference between two groups. For example, a new drug 'A' is claimed to have analgesic activity and we want to test it with the placebo. In this study, the null hypothesis would be 'drug A is not better than the placebo.' Alternative hypothesis would be 'there is a difference between new drug 'A' and placebo.' When the null hypothesis is accepted, the difference between the two groups is not significant. It means, both samples were drawn from single population, and the difference obtained between two groups was due to chance. If alternative hypothesis is proved i.e. null hypothesis is rejected, then the difference between two groups is statistically significant. A difference between drug 'A' and placebo group, which would have arisen by chance is less than five percent of the cases, that is less than 1 in 20 times is considered as statistically significant ($P < 0.05$). In any experimental procedure, there is possibility of occurring two errors.

1- Type I Error (False positive)

This is also known as α error. It is the probability of finding a difference; when no such difference actually exists, which results in the acceptance of an inactive compound as an active compound. Such an error, which is not unusual, may be tolerated because in subsequent trials, the compound will reveal itself as inactive and thus finally rejected [11]. Type I error is actually fixed in advance by choice of the level of significance employed in test [12]. It may be noted that type I error can be made small by changing the level of significance and by increasing the size of sample.

2- Type II Error (False negative)

This is also called as β error. It is the probability of inability to detect the difference when it actually exists, thus resulting in the rejection of an active compound as an inactive. This error is more serious than type I error because once we labeled the compound as inactive, there is possibility that nobody will try it again. Thus, an active compound will be lost.[11] This type of error can be minimized by taking larger sample and by employing sufficient dose of the compound under trial.

Level of Significance

If the probability (P) of an event or outcome is high, we say it is not rare or not uncommon. But, if the P is low, we say it is rare or uncommon. In biostatistics, a rare event or outcome is called significant, whereas a non-rare event is called non-significant. The 'P' value at which we regard an event or outcomes as enough to be regarded as significant that is called the significance level. In medical research, most commonly P value less than 0.05 or 5% is considered as significant level. However, on justifiable grounds, we may adopt a different standard like $P < 0.01$ or 1%. Whenever possible, it is better to give actual P values instead of $P < 0.05$[13]. Even if we have found the true value or population value from sample, we cannot be confident as we are dealing with a part of population only; howsoever big the sample may be. We would be wrong in 5% cases only if we place the population value within 95% confidence limits. Significant or insignificant indicates whether a value is likely or unlikely to occur by chance. 'P' indicates probability of relative frequency of occurrence of the difference by chance..

One-tailed and Two-tailed Test

When comparing two groups of continuous data, the null hypothesis is that there is no real difference between the groups (A and B). The alternative hypothesis is that there is a real difference between the groups. This difference could be in either direction e.g. $A > B$ or $A < B$. When there is some sure way to know in advance that the difference could only be in one direction e.g. $A > B$ and when a good ground considers only one possibility, the test is called one-tailed test. Whenever we consider both the possibilities, the test of significance is known as a two-tailed test. When we are not absolutely sure of the direction of

difference, which is usual, it is always better to use two-tailed test[14].. At present, most of the journals use two-sided P values as a standard norm in biomedical research[15].

Impoprtance of sample size determination

Sample is a fraction of the universe. Studying the universe is the best parameter. But, when it is possible to achieve the same result by taking fraction of the universe, a sample is taken. Applying this, we are saving time, manpower, cost, and at the same time, increasing efficiency. Hence, an adequate sample size is of prime importance in biomedical studies. If sample size is too small, it will not give us valid results, and validity in such a case is questionable, and therefore, whole study will be a waste. Furthermore, large sample requires more cost and manpower. It is a misuse of money to enroll more subjects than required. A good small sample is much better than a bad large sample. Hence, appropriate sample size will be ethical to produce precise results.

Factors Influencing Sample Size Include

1- Prevalence of particular event or characteristics- If the prevalence is high, small sample can be taken and vice versa. If prevalence is not known, then it can be obtained by a pilot study.

2- Probability level considered for accuracy of estimate- If we need more safeguard about conclusions on data, we need a larger sample. Hence, the size of sample would be larger when the safeguard is 99% than when it is only 95%. If only a small difference is expected and if we need to detect even that small difference, then we need a large sample.

3- Availability of money, material, and manpower.

Time bound study curtails the sample size as routinely observed with dissertation work in post graduate courses.

Sample Size Determination and Variance Estimate

To calculate sample size, the formula requires the knowledge of standard deviation or variance, but the population variance is unknown. Therefore, standard deviation has to be estimated. Frequently used sources for estimation of standard deviation are:

1- A pilot [16] or preliminary sample may be drawn from the population, and the variance computed from the sample may be used as an estimate of standard deviation. Observations used in pilot sample may be counted as a part of the final sample.[17]

2- Estimates of standard deviation may be accessible from the previous or similar studies,[17] but sometimes, they may not be correct.

Calculation of Sample Size

Calculation of sample size plays a key role while doing any research. Before calculation of sample size, following five points are to be considered very carefully. First of all, we have to assess the minimum expected difference between the groups. Then, we have to find out standard deviation of variables. Different methods for determination of standard deviation have already been discussed previously. Now, set the level of significance (alpha level, generally set at $P < 0.05$) and Power of study (1-beta = 80%). After deciding all these parameters, we have to select the formula from computer programs to obtain the sample size. Various softwares are available free of cost for calculation of sample size and power of study. Lastly, appropriate allowances are given for non-compliance and dropouts, and this will be the final sample size for each group in study. We will work on two examples to understand sample size calculation.

How to Choose an Appropriate Statistical Test

There are number of tests in biostatistics, but choice mainly depends on characteristics and type of analysis of data. Sometimes, we need to find out the difference between means or medians or association between the variables. Number of groups used in a study may vary; therefore, study design also varies. Hence, in such situation, we will have to make the decision which is more precise while selecting the appropriate test. Inappropriate test will lead to invalid conclusions. Statistical tests can be divided into parametric and non-parametric tests. If variables follow normal distribution, data can be subjected to parametric test, and for non-Gaussian distribution, we should apply non-parametric test. Statistical test should be decided at the start of the study. Following are the different parametric test used in analysis of various types of data.

1) Student's 't' Test

Mr. W. S. Gosset, a civil service statistician, introduced 't' distribution of small samples and published his work under the pseudonym 'Student.' This is one of the most widely used tests in pharmacological investigations, involving the use of small samples. In general, the independent sample t-test is used to compare observations from two populations. It tests if they have equal means or if the means of observations from two groups from one population are the same. The 't' test is always applied for analysis when the number of sample is 30 or less. It is usually applicable for graded data like blood sugar level, body weight, height etc. If sample size is more than 30, 'Z' test is applied. There are two types of 't' test, paired and unpaired.

When to apply paired and unpaired

ü When comparison has to be made between two measurements in the same subjects after two consecutive treatments, paired 't' test is used. For example, when we want to compare effect of drug A (i.e. decrease blood sugar) before start of treatment (baseline) and after 1 month of treatment with drug A.

ü When comparison is made between two measurements in two different groups, unpaired 't' test is used. For example, when we compare the effects of drug A and B (i.e. mean change in blood sugar) after one month from baseline in both groups, unpaired 't' test' is applicable.

2-ANOVA

When we deal with more than two populations or groups, we use Analysis of Variance (ANOVA). Similarly, paired t-test is used when a single sample of participant is measured twice on the same dependent variable. However, when the measurements are made more than two times repeatedly over a period of time on the same dependent variable repeated measure ANOVA should be used. The use of standard ANOVA method to compare group means is inappropriate in this kind of study design, as it does not consider dependencies between observations within subjects in the analysis. This test compares multiple groups at one time[18]. In ANOVA, we draw assumption that each sample is randomly drawn from the normal population, and also they have same variance as that of population. There are two types of ANOVA.

A- One way ANOVA

It compares three or more unmatched groups when the data are categorized in one way. For example, we may compare a control group with three different doses of aspirin in rats. Here, there are four unmatched group of rats. Therefore, we should apply one way ANOVA. We should choose repeated measures ANOVA test when the trial uses matched subjects. For example, effect of supplementation of vitamin C in each subject before, during, and after the treatment. Matching should not be based on the variable you are com paring. For example, if you are comparing blood pressures in two groups, it is better to match based on age or other variables, but it should not be to match based on blood pressure. The term repeated measures applies strictly when you give treatments repeatedly to one subjects. ANOVA works well

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even if the distribution is only approximately Gaussian. Therefore, these tests are used routinely in many field of science. The P value is calculated from the ANOVA table.

B- Two way ANOVA

Also called two factors ANOVA, determines how a response is affected by two factors. For example, you might measure a response to three different drugs in both men and women. This is a complicated test. Therefore, we think that for postgraduates, this test may not be so useful.

Importance of post hoc test

Post tests are the modification of 't' test. They account for multiple comparisons, as well as for the fact that the comparison are interrelated. ANOVA only directs whether there is significant difference between the various groups or not. If the results are significant, ANOVA does not tell us at what point the difference between various groups subsist. But, post test is capable to pinpoint the exact difference between the different groups of comparison. Therefore, post tests are very useful as far as statistics is concerned. There are five types of post- hoc test namely; Dunnett's, Turkey, Newman-Keuls, Bonferroni, and test for linear trend between mean and column number.[18]

Following are the non-parametric tests used for analysis of different types of data.

Softwares for Biostatistics

Statistical computations are now made very feasible owing to availability of computers and suitable software programs. Now a days, computers are mostly used for performing various statistical tests as it is very tedious to perform it manually. Commonly used software's are MS Office Excel, Graph Pad Prism, SPSS, NCSS, Instant, Dataplot, Sigmastat, Graph Pad Instat, Systat, Genstat, MINITAB, SAS, STATA, and Sigma Graph Pad. Free website for statistical softwares are www.statistics.com,http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize.

Statistical methods are necessary to draw valid conclusion from the data. The postgraduate students should be aware of different types of data, measures of central tendencies, and different tests commonly used in biostatistics, so that they would be able to apply these tests and analyze the data themselves. This article provides a background information, and an attempt is made to highlight the basic principles of statistical techniques and methods for the use of postgraduate students.

Conclusion

Biostatistics is common in medical research for methods of statistical analysis to become standard for particular types of data. The inappropriate uses of such methods may lead to wrong conclusions about the nature of differences and the relationship of factors for the outcome of interest under the comparison. Aside from the widely used procedures, such as one or two sample t-tests and some non-parametric tests, the standard ANOVA and repeated measures ANOVA have been of concern to researchers with their uses and interpretation of results, since researchers feel confused with the use of these statistical methods. Researchers should think about their studies carefully before they perform statistical analysis in terms of the method of measuring responses, independence among observations, and the use of appropriate models.

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