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THE SCIENCE AND ART OF BIOSTATISTICS: A COMPREHENSIVE OVERVIEW

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Abstract

Biostatistics is the area that involves the application of statistical methods to biological and health-related research and draw out valid conclusion from the obtained data. Statistics is essential to clinical trials and the drug development process, from trial design to protocol preparation. Providing a basic awareness of statistical concerns can help maintain the credibility of a clinical trial. Biostatistics is used at every stage of clinical research, including trial design, protocol development, data tracking and management, data processing, and trial reporting. This article deals with bringing in frame the basic biostatistical concepts that are significant and convenient in analyzing various types of data observations that includes the types of data, measure of central tendency, measure of dispersion, parametric and non-parametric test. Other parameters, such as the normal distribution, sample size computation, significance level, null and alternate hypothesis, and statistical significance test, are thoroughly discussed with appropriate illustrations. The review also mentions the software applications and websites helpful for statistical computations.

Key words: Biometry, parametric and non-parametric test, probability distribution, sample size.

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1. Introduction

Statistics involves gathering, organizing, analyzing, and interpreting data, serving as a valuable tool recognized as both a science and an art [1]. Tippett describes it as an art, emphasizing the importance of the statistician's expertise, experience, and understanding of the application field. Simultaneously, it is considered a science due to its systematic procedures with broad applicability across various fields. The realm of statistics encompasses all areas where an understanding of large numbers is beneficial. Biostatistics, an amalgamation of Biology and Statistics, finds its application in biological and medical sciences. It encompasses contributions from genetics, biology, epidemiology, and other related domains, including health, medicine, and nutrition [2]. Occasionally known as Biometrics or Biometry, it plays a crucial role in analyzing and interpreting data in these fields.

2. Utilities of Biostatistics [3]

Biostatistics find its utility in diverse areas which includes medicine, pharmacology, physiology and anatomy, public health and community medicine, etc. (Figure1)

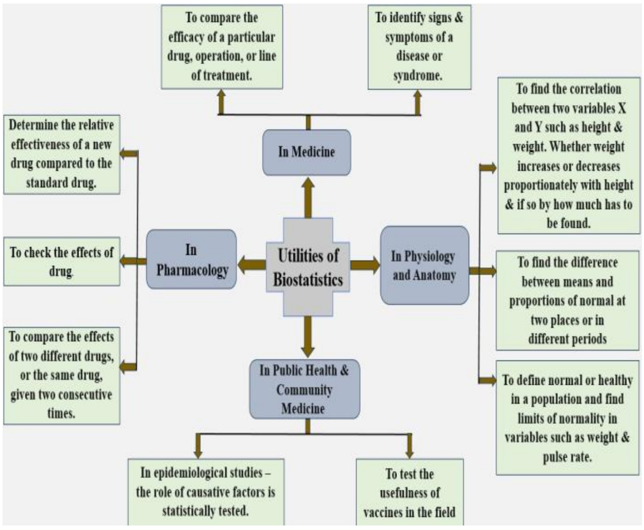

Figure 1 – Utilities of Biostatistics

3. Branches of Statistics

Statistics has been broadly divided into two major branches– Descriptive and Inferential statistics. (Figure 2)

3.1. Descriptive statistics

It is related with collection, organization, and processing of the collected data using statistical tables, diagrams and graphs. It mainly focuses on presenting the data in an understandable form which is helpful in drawing out meaningful conclusions from them. The main method of analysis include-measure of central tendency(averages), measure of dispersion. This method involves no element of uncertainty and risk.

3.2. Inferential statistics

Referred as inductive statistics, deals with drawing out prediction or inference about the population, based on statistical analysis. The statistical methods under inferential statistics includes- hypothesis testing, confidence interval and regression analysis. Since this method is based on probability it brings along, an element of risk and uncertainty.

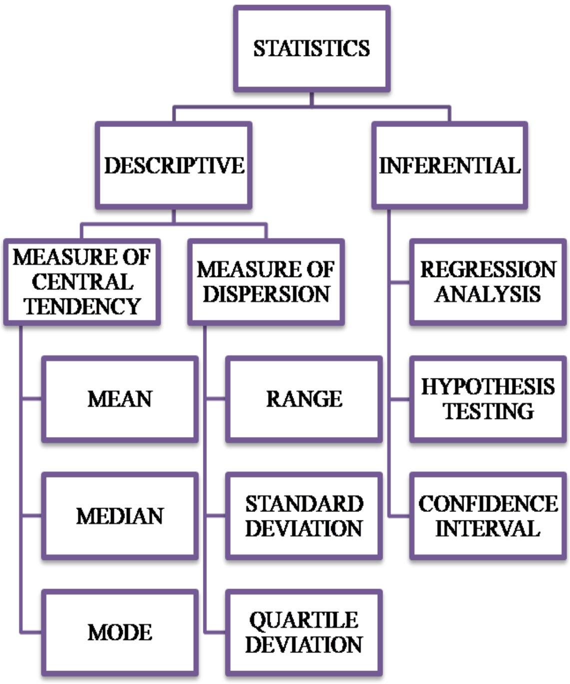

Figure 2 – Branches of Biostatistics

4. Measure of Central Tendency

Central tendency serves as a statistical measure aiming to encapsulate the essence of an entire dataset or distribution with a single value. It strives to offer a concise representation of the overall data. There are three key types of measures of central tendency -:

4.1. Mean- This represents the average value of a dataset. The quotient that results from dividing the total number of observations by their sum is the mean of a set of observations. In general, considered as the arithmetic mean or arithmetic average. Denoted by ($\bar{X}$).

$$\bar{X} = \frac{\text{Sum of all the observation}}{\text{Number of observations}}$$

The arithmetic mean of height of 9 students 60, 70, 80, 90, 90, 100, 50, 60 and 110cm is given by
Summation of individual height = 710
($\bar{X}$) = $\Sigma X/n$= 710/9 = 78.88cm

Merits-

- Robustness against Sample Fluctuations: The arithmetic mean is minimally influenced by variations within the sample.
- Well-Defined Methodology: Its calculation method is precisely defined, ensuring consistency and reliability in results.
- Simplicity and Accessibility: The concept of arithmetic mean is straightforward, making it easily comprehensible and computable even for those with limited statistical expertise.

Demerits-

- Vulnerability to Outliers: Extreme values within the dataset can significantly skew the arithmetic mean, rendering it less representative of the central tendency.
- Potential for Misleading Results: In certain scenarios, the arithmetic mean may yield results that are impractical or divergent from the actual context, leading to misleading interpretations.

4.2. Median

"H. Secrist defines the median of a series as the value, whether actual or estimated, that divides the distribution into two equal parts when the series is arranged in order of magnitude. When N observations are arranged in ascending or descending order of magnitude, the median corresponds to the middle value among them, denoted by **M**."

i. If the no of observation is odd then- **M= $[(N+1)/2]^{th}$ observation.**

Calculation of the median for the data - 520,20,340,190,35,800,1210,50,80
Following data needs to be arranged first in ascending order – 20,35,50,80,190,340,520,800,1210
Since the total no of observation is odd, therefore M= $[(9+1)/2]^{th}$ observation = 5^{th} observation
5^{th} value in the series is 190, therefore Median = 190

ii. If the no of observation is even, there shall be two middle value $(N/2)^{th}$ and $(N/2)+1^{th}$ value. The Median is then calculated as follows-

$$[(N/2)^{th} \text{ value } + (N/2)+1^{th} \text{ value}]/2.$$

Calculate the median - 25,28,29,30,30,33,33,35,40,45,46,47,48,50,52,54,53,60,65, 72
Ascending order- 25,28,29,30,30,33,33,35,40,45,46,47,48,50,52,53,54,60,65, 72
$(N/2)^{th}$ value = 20/2= 10^{th} value = 45
$(N/2)+1^{th}$ value = 11^{th} value = 46
Median= (45+46)/2 = 45.5 marks

Merits

- Insensitivity to Extreme Values: The median remains unaffected by extreme values or outliers within the dataset.
- Well-Defined Concept: The methodology for determining the median is precisely established, ensuring consistency and reliability in its calculation.
- Graphical Presentation: The median can be effectively calculated and visually represented using graphical methods, aiding in its interpretation and communication.

Demerits

- Limited Representation: In certain cases, the median may not fully represent the variability or distributional characteristics of the dataset, potentially leading to an incomplete depiction of central tendency.
- Array Issue: There can be challenges associated with determining the median when the dataset is not arranged in a proper sequence or array, requiring careful handling of data organization.

4.3. Mode

In a given set of items, the mode represents the value of the variable that occurs with the highest frequency, indicating the most common observation within the dataset. It signifies the central value around which other items are densely dispersed, reflecting the concentration or clustering of data points. Denoted by **Z** or **Mo**

Following data shows age of 10 male students suffering from DMD[4]– 6,7,8,10,12,13,14,11,8,8. The mode of data is calculated by

Arranging data in ascending order –
6,7,8,8,8,10,11,12,13,14

Most frequent occurring value i.e. mode = 8

Merits

- Simplicity: The mode offers a straightforward and easy-to-understand measure of central tendency, making it accessible to a wide range of users regardless of their statistical expertise.
- Representativeness and Stability: As the value that appears most frequently in the dataset, the mode often represents a highly typical or characteristic observation. Its stability, even in the presence of outliers or extreme values, enhances its representativeness as a measure of central tendency.

Demerits

- Sensitivity to Extreme Values: The mode can be heavily influenced by extreme values or outliers within the dataset, potentially skewing its accuracy as a measure of central tendency.
- Potential for Absurd Results: In certain scenarios, such as multimodal distributions or datasets with irregular patterns, the mode may yield unrealistic or fictitious values that do not accurately reflect the underlying data distribution.

Note

With the help of Mean and Median values, mode can be calculated using the empirical formula

Mode = 3Median- 2Mean

* Although this is appropriate in case of moderately asymmetrical frequency distributions.

5. Measure of Dispersion

The concept of dispersion complements measures of central tendency by providing insights into the variability or spread of numerical data around the average value. Dispersion reflects how individual data points deviate from the central tendency, offering a comprehensive understanding of the dataset's distribution.

Dispersion can be categorized into two types: Absolute and Relative measures of dispersion. These measures help quantify the extent of variation within the dataset and are essential for assessing the consistency and reliability of the data. (Figure 3)

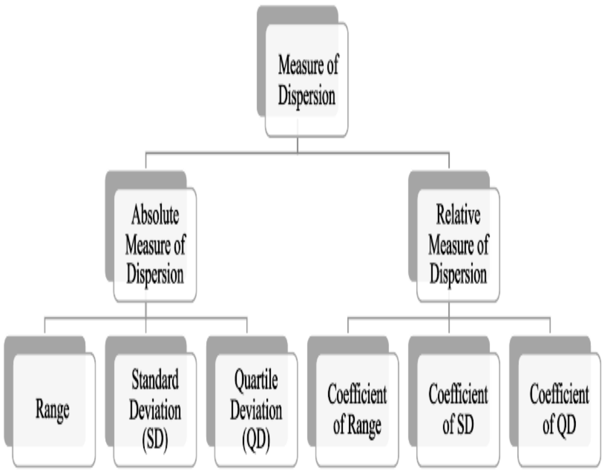

Figure 3: Measure of Dispersion

5.1. Range

It is the simplest absolute method of studying dispersion which is calculated taking the difference between value of largest and the smallest item.

Following is the data – 430,360,515,480,395,412

Largest value – 515

Smallest value – 360

Range = 515-360 = 155

Coefficient of Range= (Largest- Smallest) / (Largest + Smallest)

For the above instance -

Coefficient of Range= (515-360) / (515+360) = 0.177

5.2. Standard Deviation

It is the positive square root of arithmetic mean of the squared deviation of various values from their arithmetic mean. Denoted by **σ**. It provides a measure of the variability or dispersion of observations around the mean [5].

In order to compute the standard deviation, the following steps are followed: -

a. Calculation of the arithmetic mean ($\bar{X}$) of the series.
b. Obtaining deviation from arithmetic mean, ($d = X - \bar{X}$).
c. Square these deviations and find the sum Σd^2.
d. Divide their sum by N (no of items).
e. Obtain positive square root of the quotient to find standard deviation

Standard Deviation (σ) $=\sqrt{(\sum d^2/N)}$

Coefficient of Standard deviation- $(\sigma/\bar{X}) * 100$

5.3. Quartile Deviation

Also referred to as Semi-Inter Quartile Range. It is half of the difference between the third and first quartile. Denoted by **QD**.

$QD = (Q3 – Q1) /2$

where Q1 = size of $[(N+1)/ 4]^{th}$ item

Q3 = size of $3 [(N+1)/ 4]^{th}$ item

Coefficient of Quartile deviation-

$$\frac{(Q3 - Q1)}{(Q3 + Q1)}$$

6. Population

The term refers to an assembly of individuals who share one or more characteristics. The population in statistics refers to the total set of items used to collect study data. Represented by **N**. For instance, Delhi's population is represented by the total number of residents.

7. Sample

The sample, which represents the whole group, is a subset of the population. It ought to accurately reflect the characteristics of the whole population. Denoted by **n**. For example – 300 people from total population of Delhi represents the sample.

*Note

In situations where a researcher is unable to survey the entire population due to financial constraints, staffing requirements, or scheduling issues, a specifically chosen sample is selected to reflect the entire population.

8. Sampling Methodology

Sampling techniques are divided into two categories: probability sampling and non-probability sampling.

8.1. Probability Sampling

Every individual in the population possesses a known non-zero probability of getting chosen. Sometimes also addressed as random sampling. (Figure 4)

Merits

1. Its efficacy is independent of the possession of comprehensive knowledge about the universe.
2. Offers estimates with a high degree of precision and objectivity.
3. Sampling error can be calculated.

Limitations

1. Very high level of skill and experience is required to apply it correctly.
2. It requires a lot of time and cost of sampling is high.

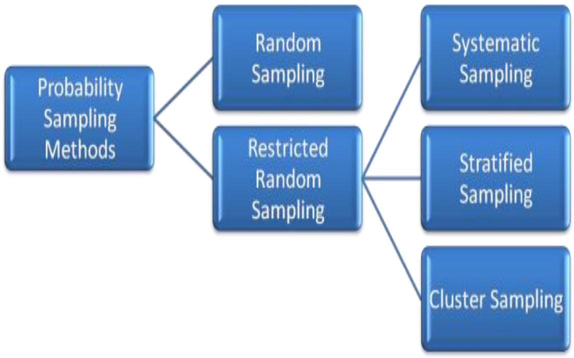

FIGURE 4: Probability Sampling methods

8.2. Non-Probability Sampling

This method selects a sample from the overall population in a non-random manner. It is uncertain, the extent to which the sample deviates from the population. Non-probability sampling is a technique wherein not every item in the universe has an equal chance of being chosen. Rather than choosing the sample based on probability, the investigator chooses it based on convenience or his judgment. (Figure 5)

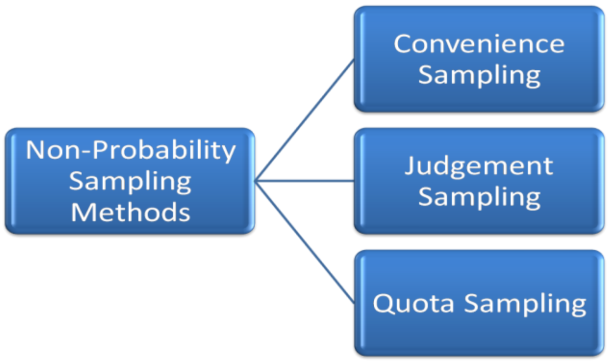

FIGURE 5 – Non- Probability Sampling methods

9. Errors in Sampling Technique

The discrepancy between the real value of the facts (population data) and the gathered data (sample data) is referred to as the Statistical error. For example there are 80 students in a class, their average in % is supposed to be

60% but if we take average marks in % of only 10 students it comes out to be 60.2%. The 0.2% is the sample error.
It is the difference between the anticipated and real values. Two categories of error exist:

9.1. Sampling Error

9.1.1. Biased Error

An error that arise on account of some biasness on part of investigator.

9.1.2. Non- Biased Error

The error occurs accidently, by chance or due to arithmetic error.
It can be reduced by taking a large sample.

9.2. Non-Sampling Error

This type of error takes place at the time of statistical data collection and is more problematic than statistical error. Statistical error can still be reduced taking large sample size whereas non-sampling error has no such alternative.

10. Sample Size Determination

A sample is a part of the universe. Studying the universe is the best parameter. However, if the same result can be achieved by sampling a portion of the universe, then the sample will be taken. This saves time, manpower, costs and increases efficiency. Therefore, appropriate sample size is of paramount importance in biomedical research. If the sample size is too small, no valid results will be obtained and the validity in such cases will be questionable and thus the entire study will be wasted. Additionally, larger samples require more cost and human resources. Registering for more subjects than necessary is a waste of money. A good, small sample is much better than a appalling, large sample. Therefore, an appropriate sample size is ethical to obtain accurate result [1].
The procedure for calculating sample size requires knowledge of the variance or standard deviation. Since the population variance is unknown, consequently, we must calculate the standard deviation. Frequently referenced resources for calculating standard deviation include:
I. From the population, a pilot sample[6] or preliminary sample may be utilized, and the variance determined from the sample can be applied to estimate the standard deviation. The final sample may include observations that were included in the pilot sample [7].
II. Estimates of the standard deviation can be retrieved from former or identical studies[7], albeit they might not always be precise.

11. Types of Probability Distribution

Probability distribution of an event shows in which way the probabilities are distributed for different variables of an event. They are of two types –

11.1. Normal probability distribution

Professor Gauss, a well-known statistician, devised this method. If the distribution of the data is symmetrical on both sides of the mean and produces a bell-shaped curve in a frequency distribution plot, it is referred to as normal or Gaussian distribution. The normal curve represents the optimum distribution of continuous variables, such as hemoglobin percentage level and blood sugar level. We can ascertain whether or not our data is regularly dispersed by directly inputting the raw data from our study into computer software and doing a distribution test. The two most important metrics that may be obtained from statistical analysis of data are the mean and the standard deviation of the mean. In an ideal normal distribution, 68.27% of all values fall between the points that are 1 SD below and 1 SD above the mean value, or ± 1 SD. Approximately 95% of values dispersed around this mean are included in the range, mean ± 2 SD, omitting 2.5% above and 2.5% below the range.[1] In an ideal distribution of values, all central tendencies—that is, mean, mode, and median—are equal within the population [2]. The distribution of sample average tends to normalize with increasing sample size, even in cases where the distribution of the original population is far from normal. This is the single most significant explanation for the normal distribution curve. There are several analytical techniques that can be used to infer assumptions about normality, such as the "t" test and analysis of variance (ANOVA). The skewness in a normal distribution is zero. The curve is positively skewed if the difference (mean–median) is positive, and negatively skewed if difference is negative. As a result, the measure of central tendency differs.

11.2. Non- normal probability distribution

When the data is skewed on either side, the distribution is not normal. It can be Poisson distribution or Binomial distribution. Poisson distribution was given by Simeon Poisson. It is a positively skewed distribution (mean> median > mode), applied when the value of p (probability of success) is very small and n (no of trial) is very large (>50). Binomial distribution also referred to as Bernoulli distribution is used in scenarios where the outcome of an experiment is in the form of Success or Failure i.e. set of only two alternatives. It is applied where no of trials are finite. When data is not normally distributed, then depending upon the nature of data different test like Wilcoxon test, Mann-Whitney U test, Kruskal-Wallis, and Friedman test are applied.

12. Hypothesis Testing

A method for assessing assumptions about the parent population from which the sample is taken is hypothesis testing. A clinical trial aims to confirm or refute a theory or belief from the outset. In statistical terminology, this assumption is called a hypothesis. The null hypothesis and the alternate hypothesis are the two sets of assumptions used in the statistical analysis test. Each research project includes both the null and the alternate hypothesis.

Null Hypothesis (Statistical hypothesis), denoted by H_0. It states that there exist no significant difference between the two groups specified in the experiment.

Alternate hypothesis (Research hypothesis), denoted by H_a or H_1. It asserts that two groups differ from one another.

For example – Two different surgical methods have been used on different patients for managing varicose veins. Method A- Vein Stripping and Method B- Ambulatory Phlebectomy [8, 9]. We want to test whether significant difference exist in the efficacy of the two surgical methods. In the study, Null hypothesis states that 'There is no significant difference in the efficacy of both the surgical methods", whereas alternate hypothesis states "Significant difference exists in the efficacy of the surgical methods used." **There is no statistically significant difference in the effectiveness of the two surgical techniques if the null hypothesis is accepted**. This indicates that the difference between the two groups was the result of chance and that both samples were taken from a single population. In the event that the alternative hypothesis is accepted i.e., the rejection of the null hypothesis is validated, there will be a statistically significant disparity in the surgical approaches' efficacy. **There are two probable faults in each experimental procedure-:**

Type I error (false positive)- also called α error, takes place when a researcher falsely rejects a null hypothesis that is true for the population. Due to its potential to affect healthcare practices, it has been determined to be more harmful [10]. For example, if we were to state that there is significant difference in the efficacy of two drugs, Drug A will reduce significant tumor in the patients as compared to Drug B (*C.reflexa)*[11], when it actually does not, this would be a **type I error**. In certain situations, making a type I error can be quite serious. For instance, if the study is continued with Drug A based on the research findings even though there was no difference in the two drugs' efficacy and the drug A has more side effects or cost patients a lot more money, we would increase healthcare cost rather than improving clinical outcomes.

Type II error (false negative)- also referred to as β error, happens when a researcher does not reject the null hypothesis even when it is wrong in the population. It is perceived to be more dangerous than the Type I error. Taking an example with a Drug that was imported for weight loss treatment in comparison to the Drug that was indigenous. Researchers would seek to show no significant difference in the efficacy of the two drugs. If, however, the imported Drug resulted in less favorable health outcomes, it would be a severe error.

13. Statistical Test

Statistical test are conducted to examine the hypothesis and draw out inference about the population. In biostatistics, there are many tests available, but the selection process is primarily based on the characteristics and type of analysis of data. The choice of statistical test should be made at the outset of the research. There are situations when we need to determine the correlation between the variables or the difference between the means or medians. A study's design can vary depending on how many groups are used. As a result, in this circumstance, we will need to choose the right test while making the most accurate decision. Invalid conclusions will result from inappropriate testing.

Statistical tests have been classified into: parametric and non-parametric. Parametric test are applied under circumstances wherein population is normally distributed or is assumed to be normally distributed. Non- parametric test are applied where population is not normally distributed. Parametric test are more powerful as they possess the ability to reject the null hypothesis when it is false.

13.1. Parametric Test -

13.1.1. Z test - The statistical test used to determine whether two population means are different when the variance is known and sample size is large. Z test is a statistical way of testing the null hypothesis when either -: (a) population variance is known and sample size is >=30 or (b) when population variance is not known but sample size is >= 30.

13.1.2. Student 't' test – When the population standard deviation is unknown and the sample size is less than 30, t-test is used to assess the statistical difference of mean values. W.S. Gosset made theoretical contributions to the "t-distribution". Since he was employed by a corporation that forbade its employees from publishing research findings under their own names, he had to publish his work using the pen name "Student." It is known as the Student t-test for this reason. The "t" test is classified in two different forms: paired and unpaired.

A) When comparing two measurements performed on the same sample after two consecutive treatments, the paired "t" test is employed. For instance, let's say we wish to assess the effects of the medication administered to eight patients (alterations in blood pressure) before and after the first month of treatment (baseline).

B) When comparing two measurements obtained from two distinct groups, the unpaired "t" test is employed. For instance, the unpaired "t" test can be used to assess the effects of drugs A and B (i.e., patient weight decrease) after one month from baseline in both groups.

13.1.3. ANOVA test – "Analysis of Variance", developed by statistician Ronald Fisher. It is an inferential statistical method to find out difference among three or more data sets or groups. It has to ability to compare multiple groups at the same time.

a) One-way ANOVA- One-way analysis of variance involves only one independent, categorical variable, or a single factor.
b) Two-way ANOVA- It is carried out when you have two independent variables. It is an extension of one-way ANOVA.

13.2. Non-Parametric Test –

13.2.1. Chi-Square test - It is a non-parametric test that is not based on any assumption or distribution of any variable. The test has been developed by Karl Pearson, which involves the calculation of a quantity, called chi-square $(\chi)^2$. Chi-square test has the most important application in the field of medical statistics as -
a. Test of Proportion
b. Test of Association
c. Test of Goodness of Fit

13.2.2. Median test – It is used for knowing whether or not two independent samples have been drawn from the population with the same median. When we are unable to satisfy the assumption and connection for using t-test, we can use median test and compare basic tendencies of the two independent samples.

13.2.3. Kruskal-Wallis test or H- test – When analyzing several independent samples, ANOVA is typically used. However, occasionally ANOVA fails to produce an adequate analysis because the sample does not meet the ANOVA's assumptions, for which another method known as Kruskal-Wallis was devised. This test aids in evaluating the alternative hypothesis—that is the means of these samples differ—against the null hypothesis, which states that "k" independent random samples originate from the same population.

13.2.4. Friedman test – It is a non-parametric test for more than two treatment groups. Basically it is used in place of the two way ANOVA test when the distribution of data is not known.

14. Biostatistics Software programs for performing statistical test

These days, the majority of statistical tests are run on computers because doing it by hand would be tedious, and time-consuming. Most widely accessed software's are MS Office Excel, Graph Pad Prism, SPSS, NCSS, Instant, Graph Pad Instat, Sysstat, Genstat, MINITAB, SAS, STATA, and Sigma Graph Pad.

Conclusion

Biostatistics is indispensable in advancing scientific knowledge, improving healthcare outcomes, and informing policies that promote public health. Its application spans various disciplines, contributing to the rigorous and reliable analysis of data in biological and health-related research. Statistical methods help identify correlations between environmental factors and health outcomes. These Statistical analysis helps healthcare providers make evidence-based decisions to enhance patient care.

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