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RESEARCH ARTICLE

"APPLIED STATISTICS IN HEALTH RESEARCH: A PRACTITIONER'S GUIDE TO GLM TECHNIQUES USING SPSS AND SAS"

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Abstract

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The general linear model (GLM) serves as a foundational statistical framework for clinical and health research, enabling rigorous analysis of treatment effects and intervention outcomes. The review critically examines the application, assumptions, and implementation of three core GLM techniques Analysis of Variance (ANOVA), Multivariate Analysis of Variance (MANOVA), and Analysis of Covariance (ANC OVA) within health research contexts. It highlights their respective purpose, from comparing group means and analyzing multivariate outcomes to adjusting for confounding covariates, while also providing practical guidance for conducting these analyses using widely adopted software packages such as SPSS and SAS. Key considerations include effect size interpretation, common violations of assumptions, and remedial strategies to maintain statistical validity.The review concludes by acknowledging the inherent limitations of GLM methods and emphasizing the importance of careful model selection, robust assumption testing, and transparent reporting to ensure findings are both methodologically sound and clinically meaningful.

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Introduction:-

Statistical analysis is pivotal in health research, it is well-known that the volume of published research, particularly in health and education, is increasing at a very rapid pace. As a consequence of the expansion of the field, qualitative and quantitative reviews of the literature are becoming more common.The relentless expansion of published health research necessitates rigorous methodological approaches to ensure valid and reliable findings. Statistical analysis forms the backbone of this empirical inquiry, and among the most powerful and versatile tools available to researchers is the General Linear Model (GLM). The proliferation of clinical research demands rigorous analytical frameworks to ensure that conclusions about treatments, interventions, and associations are both statistically sound and clinically meaningful. The General Linear Model (GLM) represents one of the most versatile and widely used families of statistical techniques in this domain. It provides a unified framework for testing hypotheses about the effects of categorical independent variables (e.g., treatment group, diagnosis) on continuous dependent variables

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(e.g., blood pressure, depression score). Its applications in health research are vast, ranging from comparing the efficacy of different drug treatments to assessing the impact of public health interventions across diverse demographic groups. This review evaluates the application of three pivotal GLM techniques in clinical research: Analysis of Variance (ANOVA), Multivariate Analysis of Variance (MANOVA), and Analysis of Covariance (ANCOVA). The objective is not merely to describe these methods but to critically appraise their use, highlighting best practices, common errors, and advanced considerations for clinical researchers. Its applications in clinical research are extensive, from evaluating the efficacy of drug treatment to assessing the impact of public health interventions across diverse populations. Closely related yet distant is the Generalized linear model (GenLM), which extends the GLM to accommodate non- normally distributed outcomes such as binary, count, or skewed data. The GenLM incorporates a random component (distribution of the outcome, e.g., binomial, Poisson, gamma), a systematic component (the linear predictor), and a link function that connects the mean of the outcome to the predictor. Logistic regression, Poisson regression, and gamma regression are common examples. Importantly, the traditional GLM can be viewed as a special case of the GenLM, using the identity link with normally distributed errors. This distinction allows researchers to select the appropriate modeling strategy depending on whether the health outcome of interest is continuous, categorical, or count-based, thereby ensuring that findings are both statistically sound and clinically meaningful.Mixed methods research integrates both quantitative and qualitative approaches within a single study to provide a more comprehensive understanding of health phenomena. Quantitative techniques such as ANOVA, MANOVA, and ANCOVA allow researchers to statistically test hypotheses and evaluate differences among groups. Qualitative methods, including interviews and focus group discussions, help explore experiences, perceptions, and contextual factors influencing health outcomes. By combining both approaches, researchers can strengthen interpretation of results and obtain deeper insights into complex health issues.

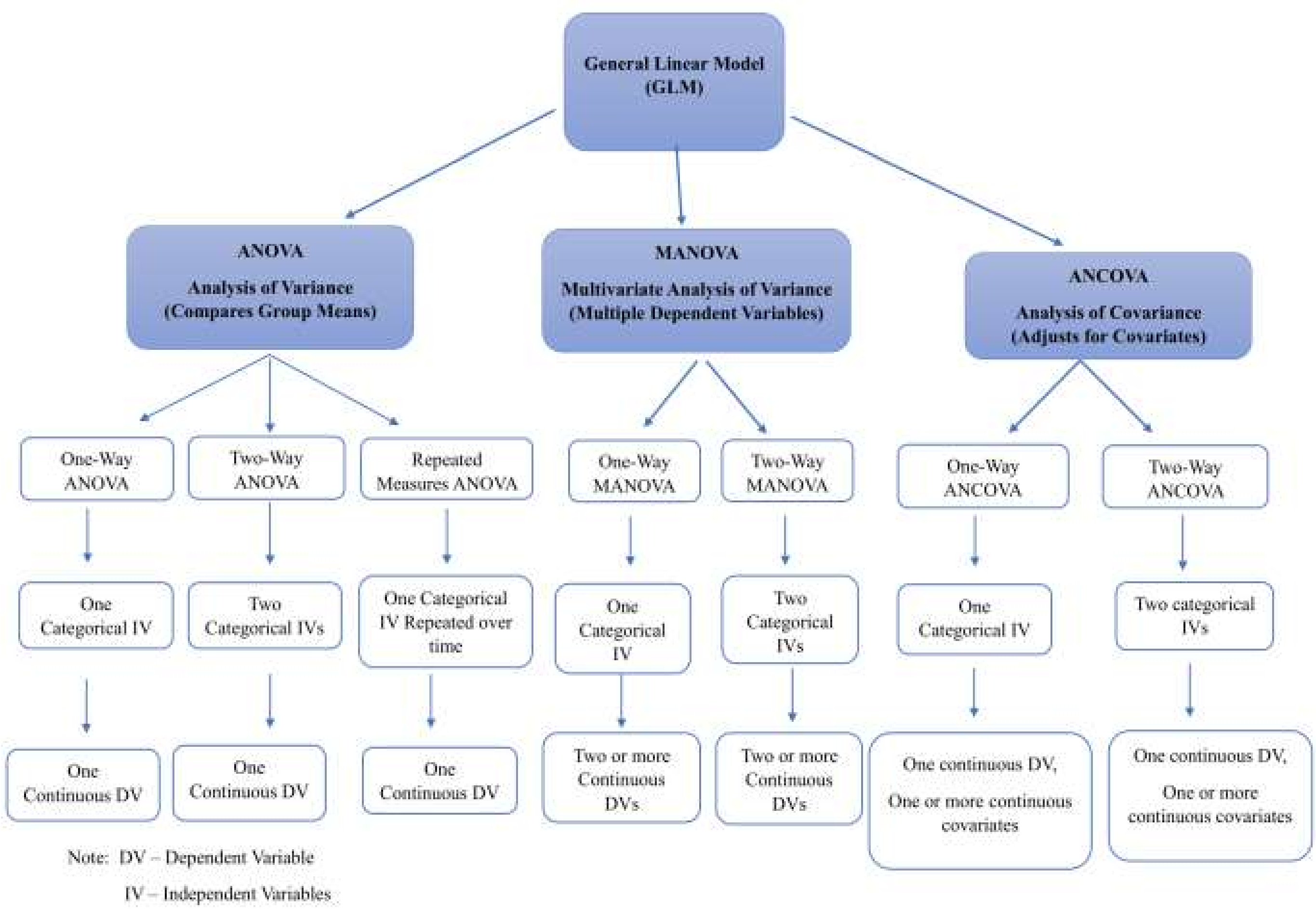

Figure 1. Conceptual framework of the General Linear Model (GLM) showing its major extensions: ANOVA,

Manova, and Ancova:-

Foundations of statistical methods:

Analysis of Variance (ANOVA):

Analysis of Variance (ANOVA) is a cornerstone statistical method for testing the hypothesis that the means of two or more populations are equal. Its fundamental principle lies in partitioning the total variance observed in a dataset into components attributable to different sources: variance between groups (explained by the independent variable) and variance within groups (unexplained error variance). The F-statistic, derived from the ratio of between-group variance (MSB) to within-group variance (MSW), is used to determine if the observed mean differences are statistically significant.

Basic Principles of ANOVA:

The fundamental principle behind ANOVA is partitioning the total variance in a dataset into components attributable to different sources. In a simple one-way ANOVA, the total variance is divided into two components.

Assumptions of ANOVA:

1. **Independence:** The observations within each group must be independent of each other.
2. **Normality:** The data within each group should be approximately normally distributed.
3. **Homogeneity Of Variance:**(Homoscedasticity)The variances of the groups should be equal. This is tested using Levene's Test
4. **Hypothesis Testing:** Null hypothesis(H0): All group means are equal.

Alternative hypothesis(H1): At least one group mean is different.

Types of ANOVA:

1. **One-Way ANOVA**: compares mean across a single factor (e.g., Intervention group)
2. **Two-Way ANOVA:** Examines the interaction and individual effects of two factors.
3. **Repeated Measures ANOVA:** This is used when the same subjects are measured multiple times under different conditions. It is particularly useful for longitudinal studies or experiments where the goal is to examine changes over time.

Assumptions:

	One-way ANOVA	**Two-way ANOVA**	**Repeated measures ANOVA**
Independence	The groups being compared must be independent of each other.	Groups must be independent of each other	Theindividual measurements are independent of each other across subjects but may not necessarily be independent within a subject
Normality	The dependent variable should be approximately normally distributed for each group.	The dependent variable should be normally distributed for each combination of factor levels.	The residuals should be approximately normally distributed.
Homogeneity of Variances	The variance within each group should be approximately the same.	Variance should be equal across groups	The variances of the differences between all possible pairs of conditions (levels) must be equal. If this assumption is violated, the Greenhouse-Geisser correction can be applied.
Interaction Effect		The model checks both the main effects of the two factors and their interaction.	

Multivariate Analysis of Variance (MANOVA):

Multivariate analysis of variance (MANOVA) extends the principles of ANOVA to scenarios with multiple, intercorrelated dependent variables. It is particularly valuable in health science, where outcomes often coexist (e.g., a psychological intervention might affect both anxiety scores and depression scores simultaneously). MANOVA tests whether mean differences among groups on a combination of these dependent variables are significant, while controlling for the correlations between them, thereby protecting against inflated Type I error rates that would occur from multiple ANOVAs.

Basic Principles of MANOVA:
MANOVA allows researchers to test whether the mean differences among the groups on a combination of dependent variables are statistically significant. It considers the interrelationships between the dependent variables and gives a fuller picture than running individual ANOVAs for each dependent variable.

Assumptions of MANOVA:
1. **Independence:** The observations must be independent of each other.
2. **Multivariate normality:** the dependent variables must have a multivariate normal distribution in each group.
3. **Use of multivariate test statistics**: includes Wilks` lambda, Pillai`s trace, Hotelling`s trace, and Roy`s largest root.
4. **Correlation between dependent variables**: accounts for the correlations between dependent variables to prevent inflated error rates
5. **Homogeneity of covariance matrices:** the covariance matrix of the dependent variables in each group.
6. **Linearity:** There should be a linear association among the dependent variables.

Types of MANOVA:
1. **One-Way MANOVA:** includes one independent categorical variable and more than one dependent variable.
2. **Two-Way MANOVA:** two categorical independent variables and more than one dependent variable, enabling analysis of interaction effects.

Assumptions:

	One Way MANOVA	**Two Way MANOVA**
Normality	The dependent variables must have a joint normal distribution.	each dependent variable must have a normal distribution for every level of the independent variables.
Homogeneity Of Covariance Matrices	There should be equality of the variance-covariance matrices between all groups.	The covariance matrices for the dependent variables should be the same across all groups.
Independence of Observations	Observations are independent.	Every observation needs to be independent.
Linearity	Linear relations between the dependent variables as well as the dependent variables and independent variables must exist.	The dependencies between the dependent variables and independent variables must be linear

Analysis of covariance (ANCOVA):
ANCOVA is a statistical method that blends ANOVA and regression analysis by incorporating continuous predictor variables, known as covariates. This allows researchers to statistically control for variables that are correlated with the dependent variable but are not the primary focus of the study (e.g., adjusting for baseline blood pressure or age when comparing a new drug to a placebo). By reducing error variance, ANCOVA increases the statistical power and precision of the analysis.

Basic Principles:
ANCOVA Scales the group means on the dependent variable by adjusting for any differences in the covariates. By doing this, the within-group variance is minimized, and it becomes easier to identify significant differences among the group means. ANCOVA is most helpful when the groups are different on the covariates because it eliminates the confounding effect of these variables.

Assumptions of ANCOVA:
1. **Independence:** The observations should be independent of one another.
2. **Normality:** The data ought to be roughly normally distributed within every group.
3. **Homogeneity of variance:** the variance of the group ought to be equal.
4. **Linearity:** there ought to exist a linear relationship between the dependent variable and the covariates.

5. **Homogeneity of regression slopes:** the slope of the relationship between the dependent variable and the covariates should be the same for all groups.
6. **Adjusting for covariates:** eliminates the effects of extraneous variables.
7. **Enhanced sensitivity:** increases statistical power by lowering the error variance.

Types of ANCOVA

1. **One-way ANCOVA:** Controls for one covariate while examining one independent variable.
2. **Two-way ANCOVA:** Controls for covariates while examining two independent variables.

Relationship Among ANOVA, MANOVA, and ANCOVA:-

Although ANOVA, MANOVA, and ANCOVA are often discussed separately, they are closely related statistical techniques within the General Linear Model (GLM) framework. Each method is designed to evaluate differences between groups but differs in terms of the number of dependent variables and the inclusion of covariates. ANOVA focuses on comparing the mean values of a single continuous dependent variable across two or more independent groups. MANOVA extends this approach by allowing the simultaneous analysis of multiple correlated dependent variables, which helps control the overall Type I error rate and provides a broader understanding of group differences. ANCOVA further expands ANOVA by incorporating one or more continuous covariates into the model, enabling researchers to adjust for potential confounding variables and improve the precision of estimated group effects. Understanding the relationships and differences among these techniques helps researchers select the most appropriate analytical method depending on their research objectives, study design, and data structure.

Comparative Analysis:

Feature	ANOVA	MANOVA	ANCOVA
Dependent variables	Single dependent variable.	Multiple dependent variables, considering their interrelationships.	Single dependent variable, adjusted for covariates.
Covariates	Not applicable.	Not applicable.	Included to control for confounding variables.
Independent variables	Categorical independent variables, can include one or more factors.	Categorical independent variables with multiple outcomes analyzed together.	Categorical independent variables, adjusted for continuous covariates.
Purpose	Compares group means to identify differences.	Examines group differences across multiple correlated outcomes.	Compares group means while controlling for confounding factors.
Complexity	Relatively simple.	High due to the need to account for correlations among outcomes.	Moderate, involves regression adjustments for covariates.
Sample size	Moderate sample size sufficient for robustness.	Larger sample sizes needed for stable multivariate estimates.	Moderate sample size needed, influenced by the number of covariates.
Assumptions	Homogeneity of variance, normality, independence of observations.	Multivariate normality, homogeneity of covariance matrices, independence.	Homogeneity of regression slopes, linearity, independence of observations.
Test statistics	F-test to compare variance components.	Wilks' Lambda, Pillai's Trace, Hotelling's Trace, Roy's Largest Root.	F-test, with adjustments for covariates.
Interpretation	Focuses on detecting group differences.	Focuses on overall group differences across multiple outcomes.	Focuses on adjusted mean differences accounting for covariates.
Applications	Treatment efficacy, demographic group comparisons, etc.	Multi-outcome clinical trials, psychological assessments, etc.	Controlling baseline differences in clinical or observational studies.

Software Implementation:
SPSS is a user-friendly, menu-driven software ideal for students and applied researchers for quick, standard analyses. SAS is a powerful, code-driven system prized in regulated industries like pharmaceuticals for its precision, ability to handle massive datasets, and guaranteed reproducibility, which is essential for clinical trials research.

Performing ANOVA, MANOVA, and ANCOVA in SPSS and SAS:
Performing ANOVA in SPSS:
SPSS (Statistical Package for the Social Sciences) is a commonly used statistical software package that offers a friendly interface to perform ANOVA in SPSS follow these steps.

1. **Data entry:** input the data into the SPSS data editor, with one column representing the independent variable (grouping variable) and another column representing the dependent variable.
2. **Select ANOVA:** go to analyze > compare means>one-way ANOVA
3. Specify variables: make the independent variable the "factor" and the dependent variable as the "dependent list."
4. **Post hoc tests(optional):** In case the results of ANOVA are significant, you may perform a post hoc test to find out which particular group means significantly differ from one another. Post hoc tests are Tukey`s HSD, Bonferroni, and scheffe.
5. **Options:** under the "Options" button, you can select descriptive statistics, homogeneity of variance tests, (e.g., Levene's test), and means plots.
6. **Run analysis:** Click "OK" to execute the ANOVA analysis.

SPSS Result Interpretation:
- ✓ f-value: indicates variance among group means.
- ✓ p-value: if less than 0.05, it suggests significant differences among groups.

Performing ANOVA IN SAS:
SAS (Statistical Analysis System) is another powerful statistical software package commonly used in research and industry. To perform ANOVA in SAS, you can use the PROC ANOVA or PROC GLM procedures

SAS
```
PROC ANOVA DATA=your_data;
CLASS independent_variable;
MODEL dependent_variable = independent_variable;
MEANS independent_variable / TUKEY;
RUN;
```

In this code:
- ✓ "PROC ANOVA" involves the ANOVA procedure.
- ✓ "DATA=your_data" specifies the dataset to use
- ✓ "CLASS independent_variable" declares the independent variables as a categorical variable
- ✓ "MODEL dependent_variable = independent_variable" specifies the ANOVA model
- ✓ "MEANS independent_variable / TUKEY" requests Tukey`s HSD post hoc test.

SAS Result Interpretation:
- ✓ Look for the F-value and p-value in the output. a low p-value (e.g., <0.05) indicates significant differences.

Conducting MANOVA IN SPSS:
To perform MANOVA in SPSS:-

1. **Data entry:** input the data into the SPSS data editor, with a single column for the independent variable (grouping variable) and several columns for the dependent variables
2. **Choose MANOVA:** analyze>general linear model > multivariate.
3. **Identify variables:** identify the independent variables as "fixed factors" and the dependent variables as "dependent variables".
4. **Options:** under the "options" button, you can choose descriptive statistics, homogeneity test (e.g., box`s M test) and post hoc tests (if applicable)
5. **Run analysis:** click "OK" to execute the MANOVA analysis.

Conducting MANOVA IN SAS:

To perform MANOVA in SAS

```
PROC GLM DATA=your data;
CLASS independent_variable;
MODEL dependent_variable1 dependent_variable2 = group;
MANOVA H=independent_variable;
RUN;
```

In this code:

- ✓ PROC GLM invokes the General Linear Model procedure.
- ✓ DATA=your_data specifies the dataset to use.
- ✓ CLASS independent_variable declares the independent variable as a categorical variable.
- ✓ MODEL dependent_variable1 dependent_variable2 = independent_variable specifies the MANOVA model with two dependent variables.
- ✓ MANOVA H=independent_variable requests the MANOVA test

Conducting ANCOVA IN SPSS:

To perform ANCOVA in SPSS:-

1. **Data entry:** input the data into the SPSS data editor, with one column representing the independent variable (grouping variable), one column representing the dependent variable, and one or more columns representing the covariates.
2. **Select ANCOVA:** Go to Analyze > General Linear Model > Univariate.
3. **Specify variables:** specify the independent variable as a "Fixed Factor," the dependent variable as the "Dependent variable," and the covariates as "covariates."
4. **Options:** under the "Options" button, you can select descriptive statistics, the homogeneity of variance test (e.g., Levene`s test), and the post hoc test.
5. **Model:** check that the model is correctly specified.
6. **Run analysis:** click "OK" to run the ANCOVA analysis

Conducting ANCOVA IN SAS:-

PROC GLM DATA=your data;

```
CLASS independent_variable;
MODEL dependent_variable=independent_variable covariate;
LSMEANS independent_variable/PDIFF ADJUST=TUKEY;
RUN;
```

In this code:

- o PROC GLM invokes the General Linear Model procedure.
- o DATA=your_data specifies the dataset to use.
- o CLASS independent_variable declares the independent variable as a categorical variable.
- o MODEL dependent_variable = independent_variable covariate specifies the ANCOVA model with one covariate.
- o LSMEANS independent_variable / PDIFF ADJUST=TUKEY requests the least squares means for the independent variable and performs Tukey's adjusted pairwise differences

Examples:

Research Scenario: Comparing two anti-hypertensive drugs on CKD progression:-

- ➢ **Population**: 200 patients with stage 3 CKD and hypertension
- ➢ **Design**: randomized controlled trial (RCT)
- ➢ **Groups:**
- ➢ **Group A:** treated with standard drug (e.g., lisinopril),
- ➢ **Group B**: treated with a new drug (e.g., losartan).
- ➢ **Primary covariate:** baseline eGFR
- ➢ **Primary outcome (time to event):** Time to a 40% reduction in eGFR or end-stage renal disease (dialysis/transplant).

- **Secondary outcome (continuous):** eGFR at 1 year, Urine Albumin-Creatinine Ratio (UACR) at 1-year, Systolic BP at 1 year.

ANOVA (analysis of variance): Research question:-
Comparing the effectiveness of three antihypertensive drugs on systolic blood pressure after one year.

Population: 240 patients with hypertension
Groups: Group A: Lisinopril Group B: Losartan Group C: Amlodipine
Variables: Independent: Drug treatment group (categorical with three levels)
Dependent: Systolic blood pressure at 1 year (continuous Interpretation:A statistically significant ANOVA result ($p < 0.05$) indicates that at least one drug group differs in mean systolic blood pressure. Post hoc tests such as Tukey's HSD can then identify which specific groups differ.

MANOVA (multivariate analysis of variance):
Research question: does the new drug have a different overall effect on the combined renal health profile (eGFR and UACR) after 1 year?
Test: one-way MANOVA
Variables:
Independent: Drug group – categorical
Dependent: eGFR at 1 year and UACR at 1 year – continuous and correlated.
Interpretation: A significant MANOVA result (e.g., $p < 0.05$ for Wilks' Lambda) indicates that the drug groups differ significantly in their combination of eGFR and UACR. This protects against Type I error that might occur from running two separate ANOVAs.

ANCOVA (analysis of covariance):
Research question: after accounting for initial kidney function, does the new drug lead to a higher eGFR at 1 year?
Test: ANCOVA
Variables:
Independent: drug group – categorical
Dependent: eGFR at 1 year – continuous
Covariate: baseline eGFR – continuous
Interpretation: The ANCOVA tests for a difference in the adjusted means of the 1-year eGFR. A significant result ($p < 0.05$) means that even after controlling for baseline kidney function, the drug group a patient was in still had a significant impact on their kidney function at 1 year.

Survival analysis (time -to- event analysis):
Research Question: Does the new drug delay the time to a 40% reduction in eGFR or end-stage renal disease compared to the standard drug?
Test: Kaplan-Meier Analysis with Log-Rank test for comparison. Often followed by a Cox Proportional-Hazards Model to adjust for other variables.
Variables:
Time: Duration from study start until the "event" (40% eGFR decline) or until the end of the study (censoring). **Event:** Did the patient experience the renal endpoint? (Yes/No). Group: Drug Group (Lisinopril vs. Losartan). **Interpretation:** The Log-Rank test compares the two survival curves. A significant p-value ($p < 0.05$) indicates a statistically significant difference in the rate at which the groups experience the renal endpoint. A Cox Model would provide a Hazard Ratio (HR). An HR of 0.5 for Losartan would mean patients on the new drug have half the risk (hazard) of experiencing kidney failure at any given time point compared to those on Lisinopril.

Practical Considerations and Advanced Topics:
Violations of Assumptions:
1. **Non-normality:** use non-parametric analyses like the Mann-Whitney or Kruskal-Wallis tests if the data does not have a normal distribution. As an alternative, data transformations, like the logarithmic transformation, could aid in data normalization.
2. **Heterogeneity of variance:** Welch`s ANOVA or Brown-Forsythe test are more resilient to breaking this assumption, thus use them if the group variances are not equal. Use techniques like Games-Howell for post hoc testing that don`t presume equal variances.

3. **Non-linearity:** if the ANCOVA connection between the dependent variable and the covariates is not linear, think about utilizing non-linear regression techniques or including polynomial terms in the model.
4. **Homogeneity of regression slopes:** if the slope of the relationship between the covariates and the dependent variable is not equal for different groups in ANCOVA, then homogeneity of regression slopes is not satisfied. This may be verified by adding an interaction term for the independent variables and the covariate in the model. When the interaction is statistically significant, ANCOVA may not be used, and other methods like separate analysis of the groups the use of more involved models, have to be employed.

Effect size measures:

1. **Eta-squared (η^2):** proportion of total variance in the dependent variable explained by the independent variable.

Formula: $\eta^2 = SS_{between} / SS_{total}$

2. **Partial eta squared ((ηp^2):** proportion of effect + error variance that is attributable to the effect.

$$\eta \mathbf{p2} = \frac{\mathbf{SSeffect}}{\mathbf{SSeffect + MSerror}}$$

3. **Cohen`s f:** another way of expressing effect size for ANOVA, related to η^2

$f = \sqrt{\eta^2}/1-\eta^2$

Practical Applications in Health Research:

Method	Type of study	Objective	Health research examples
ANOVA	Cross- sectional study	To test if there are any statistically significant differences between the means of three or more independent groups	Comparing mean pain scores patients receiving three different analgesic medications
One-Way ANOVA	Experimental study (RCT)	To determine if a single independent variable (factor) with three or more levels has a significant effect on a continuous dependent variable.	Evaluating effect of three dietary plans (e.g.; low-carb, low-fat, mediterranean) on average weight loss.
Two-Way ANOVA	Observational study	To assess the main effects of two independent variables on a dependent variable.	Analyzingthe combined effect of smoking status (smoker/nonsmoker) and genetic risk (present/absent) on mean tumor size.
Repeated measures ANOVA	Longitudinal study	To compare means of the same subjects under three or more different conditions or over time, accounting for within-subject correlation	Assessing changes in depression scores in patients measured before, during and after a 12-week therapy program.
MANOVA	Case control study	To test for significant differences between groups on a combination of two or more related dependent variables.	Comparing patients with 2 diabetes to healthy controls on a set of outcomes: fasting glucose, insulin resistance, and BMI.
One-Way MANOVA	Cross sectional study	To test the effect of one independent variable with multiple levels on multiple dependent variables.	Comparing two physiotherapy techniques on a combination of outcomes: range of motion, muscle strength.
Two-Way MANOVA	Cohort study	To test the main and interaction effects of two independent variables on multiple dependent variables.	Investigating the interaction between age group (young/old) effects of two independent variables on multiple dependent variables.
ANCOVA	Quasi experimental study	To compare group means on a dependent variable after statistically controlling for the effect of one or more continuous	Comparing the effectiveness of two wound care protocols On healing time, while controlling for the initial wound size.

		covariates (confounding variables)	
One-Way ANCOVA	Longitudinal study	To compare the means of three or more groups on a DV after adjusting for the effect of a pre-test or confounding covariate.	Comparing the impact of three antihypertensive on final blood pressure, while adjusting for baseline blood pressure.
Two- Way ANCOVA	Cohort study	To examine the main and interaction effects of two categorical independent variableson DV while controlling for one or more covariates.	Analyzing the interaction between a drug treatment and patient sex on cholesterol levels, while controlling for dietary quality.

Conclusion:-

The General Linear Model (GLM) serves as a powerful and indispensable statistical framework for clinical and health research. Its core techniques ANOVA, MANOVA, and ANCOVA provide researchers with robust methods for comparing group means, analyzing multivariate outcomes, and adjusting for confounding variable, respectively. The successful application of these models is contingent upon careful adherence to their underlying assumptions, appropriate model selection, and the use of complementary software. While SPSS offers an accessible point and click interface suitable for standard analysis, SAS provides a powerful, code driven environment essential for ensuring precision, reproducibility, and handling complex analysis in regulated research.

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