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Multiple comparison test by Tukey's honestly significant difference (HSD): Do the confident level control type I error

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Abstract
Examining a huge amount of data is a typical issue in any research process. However, different statistical processes and techniques play essential role to derive a meaningful conclusion from the presented enormous data. Control of type I error is highly essential for a researcher or statistician while dealing with comparisons test with more than two variables. Multiple testing statistical tests provides a structural system and minimizes the error rate by helping to derive meaningful accurate conclusions. Among the different multiple test procedures Tukey's honestly significant difference test (Tukey's HSD) is most common and popular techniques. The main objective of this study was to explore how significantly selection of confidence level or error rate can affect the rate of committing type I error while drawing conclusion. The effect of committing type I error with selection of confidence level or error rate was explored with citing suitable case study in a special education setting. The case study focuses cognitive effect of music on growth and enhancement of the motor behavior (running, jumping and sliding) of children with mild intellectual disability enrolled in the special school setting. ANOVA test was performed and the significance of selection of individual confidence level and simultaneous confidence level) in Tukey's HSD test was described.

Keywords: Experimental design, P value, significance level, Tukey's HSD, statistical analysis, type I error, ANOVA, special education

1. Introduction

A common problem inside the sciences and research is to examine numerous treatments to figure out which, if any, produce a superior result [1]. A crucial and important tool which helps the researchers to carry out studies on more than one experimental groups and control groups is the Analysis of Variance (ANOVA).The analysis of variance (ANOVA) gives a significant result, which identified the difference between the groups more probably it indicates that at least one group differs from the other groups. However, the test don't inform on the pattern of differences between the means [2].

The major setback associated with ANOVA is that it only compares the means between groups and the results concluded if all group means under consideration are equal or not, but does not categorize the exact information such as which particular pairs of means are significant. In order to get the accurate and specific pattern of difference between means, the ANOVA is often equipped with specific test specific compares the two means between the pairs or groups (the called pair wise comparisons) [3, 4].

Multiple comparisons of means allow to examine which means are different and to estimate by how much they are different. The statistical difference between means can be assessed by using statistical tools operates on fundamental concept of statistics using set of confidence intervals or hypothesis tests or both [5, 6]. These types of pair wise comparison tests are called multiple comparison techniques and the most frequently technique was developed by Tukey and named as the honestly significant difference (HSD) test. The main idea of the HSD is works on the principle of computation of the honestly significant difference between two

means using a statistical process. The process gives the exact information and identifies the major difference between means of a set of group from the population under consideration [7].

Whenever a researcher has more than two comparisons to test, control of the Type I error rate becomes a concern. The analysis of variance (ANOVA) certainly helps the researcher to identify existence of significant effect however it was created problem to control the type I error while dealing with multiple t-tests with multiple groups. Even if Fisher recommended using a more stringent alpha while performing his Least Significant Difference Procedure (LSD) but researchers finds the LSD process inadequate control of Type I error [8, 9]. The problem associated with fisher method and the hunger to get better statistical methods leads to development of several multiple comparison methods. Out of these several developed methods, the most popular process for comparison of means between the pairs or groups in a population is the Tukey's Honestly Significance Difference Procedure (HSD) [10]. From this it can be clearly identified the role of selection of significance level (alpha value) is highly crucial in multiple comparison test. Tukey's HSD is probably the most recommended and used procedure for controlling Type I error rate when making multiple pair wise comparisons. The purpose of this study was to compare observed Type I errors with nominal confidence level of 90 %, 95 % and 99% in special education setting.

2. The Multiple comparisons method
2.1 The concept of multiple comparisons method
Multiple comparison methods are used to investigate differences between pairs of population means. In specific it investigates difference between subsets of population means using sample data. A set of confidence level is used to assess the statistical difference between the means of groups. The confidence intervals allow to evaluate both statistical significance and the practical significance of differences among means. Based on the basic characteristics of the hypothesis concept, the null hypothesis of no difference between means is rejected if zero is not contained in the confidence interval [11].

2.2 Types of multiple comparison method and its use
The selection of the appropriate multiple comparison method depends on the desired inference. It is always not useful to use the Tukey all pair wise approach when other tests are suitable for study. Tukey confidence intervals are always wider and the hypothesis tests are less powerful for a particular family error rate. Dunnett is preferable when the study has a control group and several treatment groups and the aim is to compare the treatment group with the control group. In this instant, MCB is always preferable as compared to Dunnett if the requirement is to identify the groups that are the best. The choice of Tukey versus Fisher's LSD methods mainly depends on the error rate i.e. based on requirement of error rate; it might be family error rate individual error rate [12].

Table 1: Characteristics and advantages of different multiple comparison methods [11, 13, 14].

Method	Normal Data	Strength	Comparison with a Control	Pair wise Comparison
Tukey	Yes	Highly recommended test when doing all pair wise comparisons.	No	Yes
Dennett	Yes	Most influential test when comparison is with a control.	Yes	No
Hsu's MCB method	Yes	The most powerful test when comparison is done with the group with the highest or lowest mean to the other groups.	No	Yes
Games- Howell	Yes	Used when equal variances is not primary criteria	No	Yes

3. Tukey HSD Test
The Tukey HSD ("honestly significant difference" or "honest significant difference") test is a statistical tool used to determine if the relationship between two sets of data is statistically significant – that is, whether there's a strong chance that an observed numerical change in one value is causally related to an observed change in another value. In other words, the Tukey test is a way to test an experimental hypothesis. The Tukey test is invoked if the interaction among three or more variables is mutually statistically significant, which unfortunately is not simply a sum or product of the individual levels of significance [15, 16].

Tukey's method is used in ANOVA to create confidence intervals for all pair wise differences between factor level means while controlling the family error rate to a specified level. It is essential to carefully evaluate the family error rate when making multiple comparisons because the chances of making a type I error for a series of comparisons is greater than the error rate for any one comparison alone. To reduce the higher error rate, Tukey's method calculate and adjusts the confidence level for all individual comparisons that produces the family error rate so that the resulting simultaneous confidence level is equal to the specified value [17, 18].

3.1 Need of Tukey HSD Test
Initially the results of multiple test studies conclusion were drawn based on the alpha value and independent P value. John Tukey later realized the drawback associated with this

process and came up with the test that bears his name. The Tukey's test compares the differences between means of values rather than comparing pairs of values. The value of the Tukey test is given by taking the absolute value of the difference between pairs of means and dividing it by the standard error of the mean (SE) as determined by a one-way ANOVA test. The SE is in turn the square root of (variance divided by sample size) [19].

The Tukey test is a post hoc test in that the comparisons between variables are made after the data has already been collected.

3.2 Steps wise process to perform Tukey HSD Test
The Tukey HSD test analyzes all pair wise comparisons among means. For measurement or analysis the HSD for each pair of mean was calculated by using the below formula [20]:

$$HSD = \frac{M_i - M_j}{\sqrt{MS_w / N}}$$

Where:
1. $M_i - M_j$ is the difference between the pair of means. (To calculate this, M_i should be larger than M_j)
2. MS_w is the Mean Square Within, and N is the number in the group or treatment.

Steps involved
Step 1: Perform the ANOVA test. Assuming your F value is significant; run the post hoc test

Step 2: Choose two means from the ANOVA output. Note the following:
- Means,
- Mean Square Within,
- Number per treatment/group,
- Degrees of freedom within.

Step 3: Perform the Tukey's HSD statistics by using the above formula.

Step 4: Determine the score reported in Tukey's critical value table.

Step 5: compare the values obtained in step 3 and step 4. If the calculated value from Step 3 is larger than the value obtained in step 4 then it can be concluded that means are significantly different.

4. Case study for control of type I error (with respect to significance level) while designing HSD experiment using statistical tools

A case study was presented for the process of performing HSD during investigation in a special education setting. The study focuses cognitive effect of music on growth and enhancement of the motor behavior (running, jumping and sliding) of children with mild intellectual disability enrolled in the special school setting located in the Ganjam District of Odisha. The case study includes sample selection, design of study, tools used and effectively shown the affect of type I error with change in significance level. The case study explains the experiment wise Type I error control with respect to different significance level.

4.1 Selection of Sample

The population of the study will consist of 10 intellectual disable special school children with mild mental retardation. All intellectual disable students belong to mild category. The study will be conducted with the help of "BALVIKAS" located near Kodola town of Ganjam district Odisha, INDIA. The study also considers only male student as an integral part of the study. These students were selected based on their Intelligence Quotient (IQ) and between age group 8-15 years [20].

4.2 Tools used

The tools used are mainly the available tools used by the professionals in the field of statistics and intellectual disability. The motor skill of individual subject was assessed in terms of score (percentage) and appropriate tools will be identified, developed and administered. The main tool used for the evaluation of this study was statistical software Minitab 17.3.0.

4.3 Scoring and interpretation

First, basic information (such as name, age, sex, IQ and level of class) were collected for all the children with mild mental retardation enrolled in the study. Then the children were subjected to the matching procedures on the variables mentioned (i.e. age, sex and IQ). Thus, a total of 10 intellectual disable children with mild mentally retardation are selected for each group. In the similar way 3 groups are created and totally 30 intellectual disable children with mild mentally retardation are enrolled. The age limit for the children was 8 -15 years and the IQ considered was 40-50 for group 1, 50-60 for group 2 and 60-70 for group 3 respectively.

After the identification of the sample, the motor skills (running, jumping and sliding) of individual subjects were assessed by using the motor skill assessment scale. The observations were recorded on the observation sheet of the record booklet and scoring was done in the assessment manual. The Numerical score and raw score of all domains were calculated by using the assessment manual.

Item No.	Domains of motor skill development Running/Jumping/Sliding	Assessment
A.	Not Responded (N)	
B.	Occasionally Responded or try to respond (O)	
C.	Responded well (R)	

5. Presentation of the case study for control of type I error (with respect to significance level) while designing HSD experiment

As per the study design the motor skill development scores after effective use of music for ten students in each group were collected after implementation of music. The details of scores of the students with their mean, minimum and maximum marks obtained are presented in the subsequent section.

5.1 Effect of type 1 error in HSD experiment consisting of 3 groups with different significance level (90, 95 and 99 %)

The effect of type I error in HSD experiment was performed by taking three groups. The details of numerical scores of gross motor skills obtain by the intellectual disability students consisting of 3 groups (group 1, group 2 and group 3) with their mean, minimum and maximum scores obtained are presented in Table 1.

Table 1: Individual scores obtained by students (%) consisting of 3 groups (group 1, group 2 and group 3)

Students	Individual scores obtained by students (%) consisting of 3 groups		
	Group 1	Group 2	Group 3
1	82.64	65.83	48.21
2	66.69	75.12	44.26
3	62.03	54.22	65.42
4	80.02	41.36	55.22
5	61.24	42.28	42.22
6	76.32	51.26	56.23
7	62.21	38.24	41.25
8	48.22	45.21	44.82
9	53.21	65.24	52.26
10	44.45	56.84	41.26
Mean	63.703	53.56	49.115
Min	44.45	38.24	41.25
Max	82.64	75.12	65.42

5.2 HSD experiment consisting of 3 groups with different confidence level (90, 95 and 99 %) or error rate (1, 5, and 10 %)

The one-way analysis of variance (ANOVA) is used to determine whether the mean of a dependent variable is the same in two or more independent variable. ANOVA only helps to determine that at least two groups were different in the studied population. In the discussed case study one way ANOVA was applied with significance level of 0.05 (α) to identify which group is different from all the three studied groups (three groups i.e., scores obtained by group 1, group 2 and group 3).

Both one-way analysis of variance (ANOVA) and the Tukey HSD test were performed simultaneously by using statistical software Minitab 17.3.0. However, during Tukey HSD test different error rate of comparison or confidence level (90, 95 and 99 % Cl) were taken to compare the mean score of three groups. The interval plot of scores vs group (with 95 % CI of mean) from one way ANOVA and Tukey simultaneous with different significance level (90, 95 and 99 %)) were plotted. The details of ANOVA summary and HSD test was presented in Table 2 and 3

5.3 One-way ANOVA: Score versus Group
The one way ANOVA test was performed to identify whether the specific groups were significantly different from each other or not. The following method was adopted to perform the ANOVA study.

6. Method
Null hypothesis: All means are equal
Alternative hypothesis: At least one mean is different
Significance level: $\alpha = 0.05$
(Equal variances were assumed for the analysis)

Table 2: Analysis of variance table

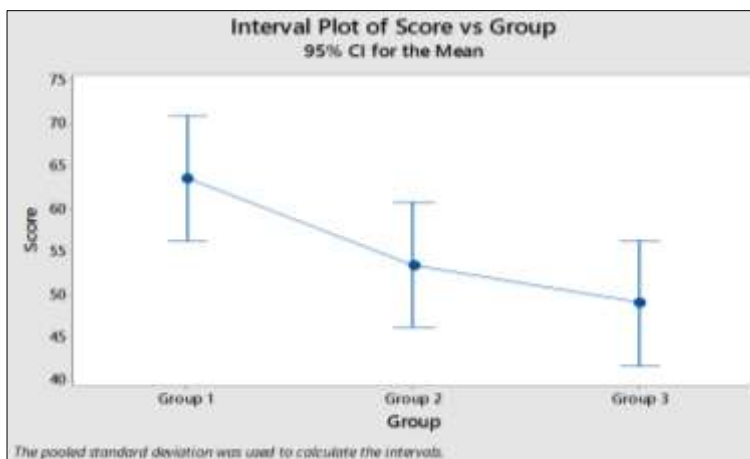| Source	DF	Adj Sum of square	Adj Mean square	F-Value	P-Value
Group	2	1118	559.1		
Error	27	3451	127.8	4.37	0.023
Total	29	4569	-		

Fig 1: One way ANOVA plot of score vs group (3 groups) with 95 % CI for the mean

The result of one way ANOVA reveled that P value was found to be 0.023 which signifies that there is significant difference exist in the scores obtained in different groups. The one way ANOVA plot in Fig. 1 also signifies that the mean values of all three groups interval distributed far from their respective means.

From Table 1 and Fig 1, it was identified that ANOVA detects a significant effect of scores between the groups. However, there is no information which specific population groups differ significantly from one another. For this purpose, there is a requirement to test the differences between pairs of groups. In this case the pair wise multiple comparisons tests, also called post hoc tests, are the right tools to address this issue. Thus, Tukey pair wise comparisons test was selected and the difference in means of the group was determined.

6.1 Tukey Pair wise Comparisons
As discussed earlier Tukey HSD test was performed with different error rate of comparison (1,5 and 10 %) or confidence level (90, 95 and 99 %) to compare the mean score of three groups. The Tukey simultaneous with different error rate (90, 95 and 99 %) was presented in Table 3, Table 4 and Fig 2.

Table 3: Grouping information (3 groups) using the Tukey Method with 90 %, 95 %, and 99 % confidence

Group	N	Mean	Grouping
Grouping information using the Tukey Method and 95% confidence			
Group 1	10	63.70	A
Group 2	10	53.56	A B
Group 3	10	49.11	B
Grouping information using the Tukey Method and 99% confidence			
Group 1	10	63.70	A
Group 2	10	53.56	A
Group 3	10	49.11	A
Grouping information using the Tukey Method and 90% confidence			
Group 1	10	63.70	A
Group 2	10	53.56	A B
Group 3	10	49.11	B

Note: Means that do not share a letter are significantly different.

The results of the Tukey's test are included in the grouping information table (Table 3), which highlights the significant and non-significant comparisons. The information captured in the above table specifically grouping information table helps to rapidly determine whether any significant difference between means of any pair of groups subsist or not. The

grouping column presented with alphabetical letters that group the factor levels. Groups that do not share a letter have a mean difference that is statistically significant. Table 3 shows that in 95 % confidence only group 2 shares both letter A and B. However, it was observed that group 1 and 3 do not share a letter, which indicates that group 1 and 3 are significantly different. It also provides evident that group 1 has a significant higher mean as compared to group 3. Same

trend is also observed for in 90 % confidence interval or error rate. However, in 99 % confidence it was observed that all the three groups are significantly different. It is might be due to selection of 1 % error rate. The selection of 99 % confidence level or 1 % error rate makes the test more sensitive to detecting differences, thus a small change in means shows a significant difference.

Table 4: Tukey simultaneous tests for differences of means for 95 %, 90 % and 99 % confidence level

| Difference of Levels	Difference of means	SE of difference	95 % CI	T-value	Adjusted P-value
Tukey simultaneous tests for differences of means for 95 % confidence level					
Group 2 - Group 1	-10.14	5.06	(-22.69, 2.41)	-2.01	0.130
Group 3 - Group 1	-14.59	5.06	(-27.14, -2.04)	-2.89	0.020
Group 3 - Group 2	-4.44	5.06	(-16.99, 8.10)	-0.88	0.658
Individual confidence level = 98.04%					
Tukey simultaneous tests for differences of means for 90 % confidence level					
Difference of Levels	**Difference of means**	**SE of difference**	**90 % CI**	**T-value**	**Adjusted P-value**
Group 2 - Group 1	-10.14	5.06	(-20.98, 0.69)	-2.01	0.130
Group 3 - Group 1	-14.59	5.06	(-25.42, -3.76)	-2.89	0.020
Group 3 - Group 2	-4.44	5.06	(-15.28, 6.39)	-0.88	0.658
Individual confidence level = 95.87%					
Tukey simultaneous tests for differences of means for 99 % confidence level					
Difference of Levels	**Difference of means**	**SE of difference**	**99 % CI**	**T-value**	**Adjusted P-value**
Group 2 - Group 1	-10.14	5.06	(-26.23, 5.94)	-2.01	0.130
Group 3 - Group 1	-14.59	5.06	(-30.68, 1.50)	-2.89	0.020
Group 3 - Group 2	-4.44	5.06	(-20.53, 11.64)	-0.88	0.658
Individual confidence level = 99.63%					

(A)

(B)

(C)
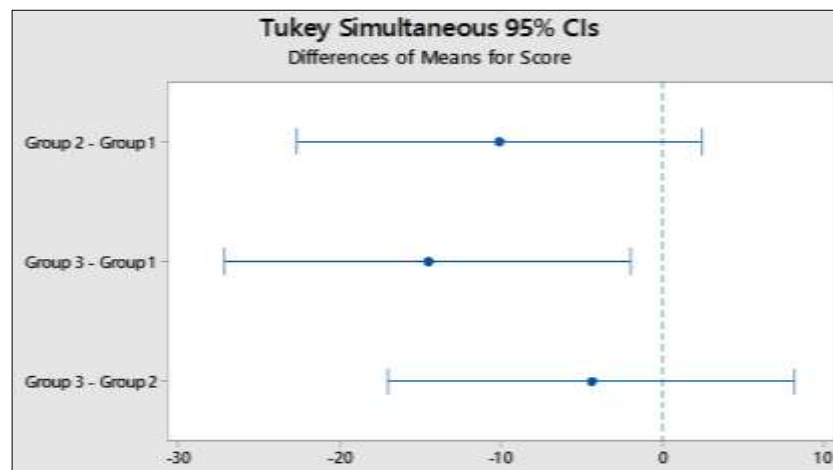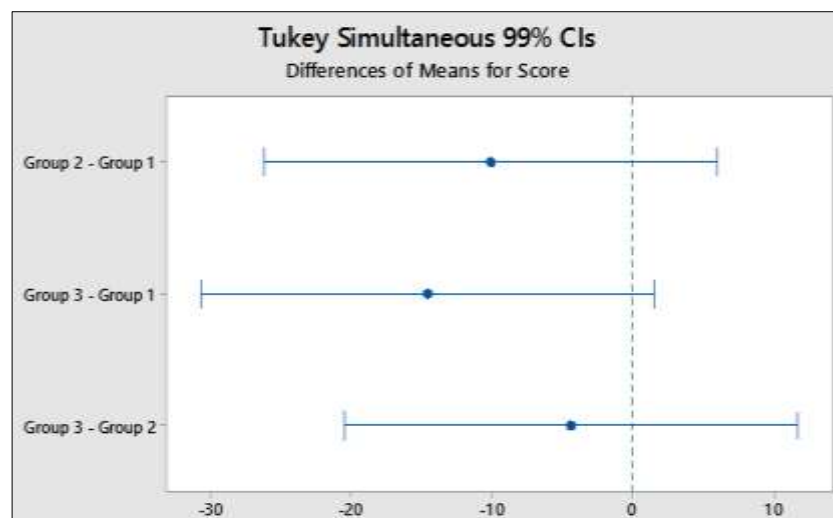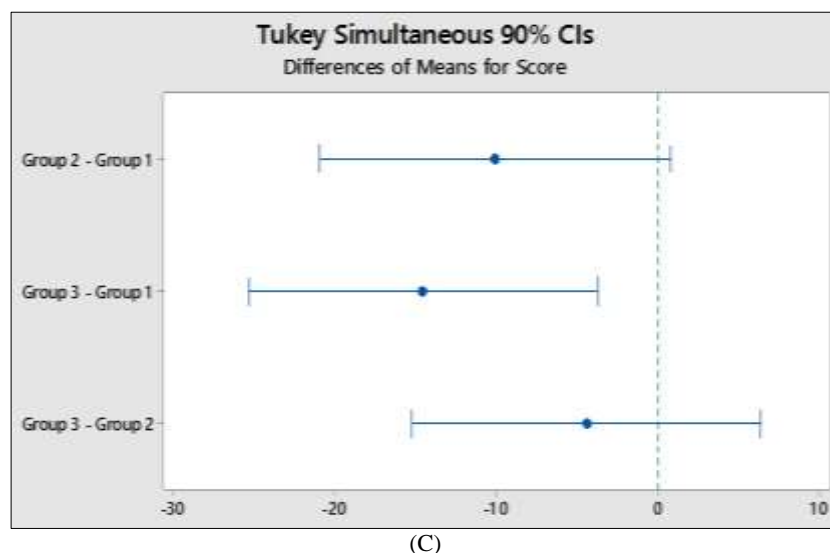
Fig 2: Tukey Simultaneous test for differences of mean of score for 3 groups with (A) 95% CI (B) 99% CI and (C) 90% CI

7. Observation and discussions

The confidence intervals in the Table 4 and Fig 2 for the above case study indicate the following:

1. The 95% simultaneous confidence level indicates that we are 95% confident that all of these confidence intervals contain the true differences. The same is true for 90% and 99 % confident level.
2. The 95 % confidence level for the difference between the means of group 1- group 2 and group 2 – group 3 extends from -22.69 to 2.41 and -16.99 to 8.10 respectively. This range include zero, which indicates that the difference between these means is not statistically significant.
3. 95 % confidence level for the difference between the means of group 1- group 3 extends from -27.14 to -2.04. The confidence levels for this range does not include zero, which indicates that the differences are statistically significant.
4. Each individual confidence interval has a confidence level of 98.04%. This result indicates that 98.89% confident that each individual interval contains the true difference between a specific pair of group means. The individual confidence level for each comparison produces the 95% simultaneous confidence level for all three comparisons.
5. The 90 % confidence level for the difference between the means of group 1- group 2 and group 2 – group 3 extends from -20.98 to 0.69 and -15.28 to 6.39 respectively. This range include zero, which indicates that the difference between these means is not statistically significant.
6. 90 % confidence level for the difference between the means of group 1- group 3 extends from -25.42 to -3.76. The confidence levels for this range does not include zero, which indicates that the differences are statistically significant.
7. The 99 % confidence level for the difference between the means of group 1- group 2, group 1 group 3 and group 2 – group 3 include zero in their respective range, which indicates that the difference between these means is not statistically significant.
8. However, from Table 4, it was observed that at 99% confidence level the adjusted P vale for group 1 – group 3 was 0.020 which was lesser than selected significance level. The P value indicated that there is significant difference exist between group 1 – group 3. The same result also reflected in the grouping data.

9. The conflict results obtained with 99 % confidence level for group 1-group 3 is might be due to improper selection of error rate which produces type I error highly prominent in multiple comparisons test like Tukey's test.
10. The experiment-wise error rate represents the probability of a type I error (false positive) over the total family of comparisons. The simultaneous confidence level is based on both the individual confidence level and the number of confidence intervals. In 99 % confidence level the individual confidence level also found to be 99.63%. The observed type I error with 99 % simultaneous confidence level is resulted due to the high individual confidence level.
11. In post hoc tests like Tukey's HSD, simultaneous confidence level was used rather than an individual confidence level. The simultaneous confidence level applies to the entire family of comparisons. With a 99% simultaneous confidence level, it can be 99% confident that all intervals in the set of comparisons contain the actual population differences between groups. However, getting a 99 % confidence in an experiment is highly difficult. This makes the tests more sensitive to commit error and this is the probable reason that leads to committing a type I error as compared to 90 % and 95 % confidence level.

8. Recommendations

In the above case study one-way analysis of variance (ANOVA) followed by Tukey HSD test was performed to identify differences between the means of pairs of groups. Three error rates are studied and committing of type I error was evaluated for each error rate. From the result it was identified that with 90% and 95 % error rate or confidence level the probability of committing type I error is less. However, a type I error was observed with 99 % error rate. The possible reason of the observed type I error was identified and based the observed data and the results following recommendations are proposed

1. Error rate or confidence level should be taken carefully before commencing Tukey HSD test. Probability of committing type I error increases with improper selection of confidence level.
2. The size of population always plays an important role. Sample size is also a significant predictor; as sample size increases the difference decreases. Thus, it is always

advisable to select larger number of populations in the pair or group to minimize the type I error rate while performing Tukey HSD test.

3. The probability of committing a type I error also depends on the no of pairs taken to compare the mean. It is assumed that when the number of groups increases the difference between experiment wise errors proportions also increases. Thus, it is also advisable to perform Tukey HSD test with suitable numbers of pairs or groups to compare the mean. The selection of error rate or the confidence level plays a significant role while performing HSD test in pairs or groups.

9. Conclusion

The selection of statistical parameters plays a significant role for statistical analysis of any data with more than two comparisons. In fact, the search of appropriate conditions such as level of significance may be considered helpful for the researcher or statistician to draw meaningful conclusion. However, the research output also significantly depends on type I error. In statistical hypothesis testing, a type I error is the rejection of a true null hypothesis (also known as a "false positive") during drawing conclusion. Controlling appropriate error rates is highly essential in order to protect against wrong conclusions.

In the present study the importance of selection of error rate or confidence level was explained with a study conducted with the help of special school of BALVIKAS situated in Kodola, Ganjam, India with ten mild special intellectual disable students in three groups. The study was conducted with the obtained raw data and sequential approach was performed by using Tukey's HSD test to find the suitable confidence level to minimize the type I error. The one way ANOVA study was conducted and Tukey's HSD test was performed with different error rate. The error rate was calculated and then significant of different significance level with respect to Tukey's HSD test was explained by using P value of ANOVA test and grouping information obtained from the Tukey test. The case study demonstrated that the confident level controls the type I error in Tukey's HSD test. In the presented case study the conflict results obtained with 99 % confidence level for group 1-group 3 is resulted due to the high individual confidence level. Based on the result, recommendations are proposed to minimize type I error while performing Tukey's simultaneous test.

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Competing interests
Authors have declared that no competing interests exist.

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