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Lecture - 09 t-tests

Welcome to the course on Biostatistics and Design of Experiments. Today we will continue on the t-test like I had mentioned that t-test does a comparison of a means.

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Compa	aring Means
The t-test allow you to determ difference in the mouns of two sample and an established value	re if there is a statistically significant samples, or between the mean of a set
T-tests are robust to non-normal data The test is more sensitive if the two groups being compared have similar standard deviations.	What tool should you use? • <u>1-sample 1-test</u> : If you want to compare the mean of your data to a known value • <u>2-sample 1-test</u> : If you want to compare the means of two samples • <u>Paired 1-Test</u> : If you are comparing the difference between two paired samples to zero.

If the mean of one sample and you have a mean of another sample, you want to know whether they come from the same population or they come from different population and so on actually. Basically, t-test compares means, there are 3 different types of t-test 1 sample t-test, 2 sample t-test and paired t-test. So, in 1 sample t-test what to you do is, you take a sample and the mean which you get compare it with the population mean and you find out whether this sample mean comes from this population or not. In a two sample t-test, you are comparing 2 sets of samples for example if I am comparing drug a and drug b performance, then I will get some mean for sample set 1 and I will get some mean for sample set 2 and I would like to see whether there is any statistically significant difference between these 2 means or not. In the paired t-test, you use the same subject for performing the experiments for example, if I am performing clinical trials and I have 10

volunteers I may give drug a to the 10 volunteers and after a few days I may give drug b to the same 10 volunteers and I will see the change. So theoretically, if a and b perform in the same way that difference should be 0 that is called the paired t-test.

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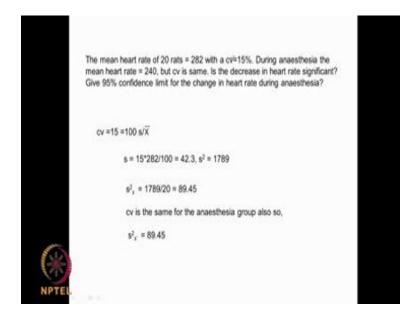


Let us continue with some problems because problems are very very important, problems give you an idea about a how to attack these type of a situations and we need to know how to use the tables, we need to know when to use a 1 sample t-test or a 2 sample t-test, when to use a 1 tailed or 2 tailed, how to create the a null hypothesis and alternate hypothesis and so on actually. The mean heart rate of 20 rats is say 282 with a coefficient variation of 15 %, what is coefficient of variation?

100 s / μ

During anesthesia the mean rate becomes 240, it looks like it is gone down, but the CV is the same. Is the decrease in heart rate significant? We need to find out whether the decrease in the heart rate is significant. Give 95 % confidence limit for the change in the heart rate that means, it is the change that is 240 - 282 or 282 - 240. What is the confidence interval? Although we say 282 - 240 the change is 42, but then there is always be going to be a region of confidence because in any mean will always have a standard deviation. Let us go about solving 1 at a time.

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So coefficient of variation is given by 15 %, 15 is equal to 100 into s the standard deviation divided \overline{X} . As you can see 282 is the mean that is \overline{X} so from there I calculate s simple. I just rearrange, so s comes out to be 42.3. Now the variance

 $s^2 = 1789$

Now I can calculate the standard error of the mean by divide by 20 here right if you remember, what is the standard error s by square root of n or the square of the standard error if the mean will be $s^{2'}n$, that is what I get it 89.45. Now CV is a same for the anesthesia group also, the standard error will be the same for the anesthesia group do you understand this so far. I know CV, I take 282 here \times so I calculate s that is the standard deviation. Now the standard error of the mean, I just have to divide by n and then later on I need to take square root, but right now we are not taking square root we leave it like that. Now CV is the same for the anesthesia group also, so standard error for the anesthesia group also will be the same ok.

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Let us formulate our hypothesis,

H₀ : μ =μ_o

that means there is no difference, in

Ha: μ< μ_ο

. If you read the problem decrease in heart rate, so we are saying $\mu < \mu_0$ So what do you do we take our t equation,

$$t = \frac{\overline{X} - \mu o}{\frac{S}{\sqrt{n}}}$$

you remember this equation right,

$$t = \frac{\overline{X} - \mu o}{\frac{S}{\sqrt{n}}}$$

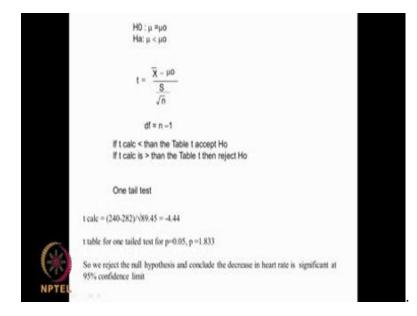
this call the standard error and mu not is the population mean and this is a sample mean t degrees of freedom will n minus 1.

df = n –1

We calculate the t and them we compare table t, if the t calculated is less than the table t we accept **Ho**, the t calculated is greater than the table t we reject the **Ho**. Here, it is a **1** tail test please remember because we are talking about this type of situation, we are not saying

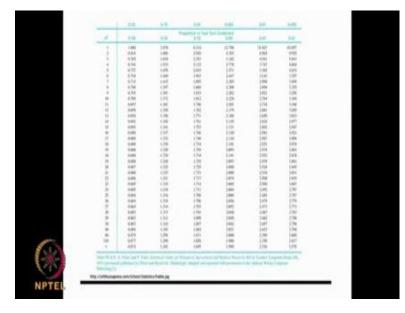
 $\mu_0 = \mu_0$ that means less or more, but we are specifically talking about μ a less than μt . So it is a 1 tail test simple.

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What we do is we t calculate I know 240 the other one is 282, if you remember we got 89.45, so square root of 89.45 is what I need put here. 240 - 282 I will get - 4.44. Now let us go to t table, with t table if I go for 1 tail test for p = 0.05, 1 tail test p = 0.05

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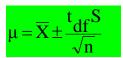


I need to look into this region. I get the 9 degrees of freedom because there are 10 rats, 10 - 1 is 9, it is 1.833. P from the table is 1.833, t calculated is - 4.44. It is greater, so we reject the null hypothesis at p = 0.05. We need to accept the alternative hypothesis that means, the heart rate has decreased because of anesthesia and this decrease is significant at 95 confidence interval. So you understand this it is very simple. We need to use this table as I said if you are talking about 1 tail test use the top numbers, if you are using 2 tail test we use the bottom number. When I said 0.05 for 2 tail I mean sorry 1 tail I used this, where has if I had said for a 2 tail I would have used this. As I said before also, 2 tail includes the outside area on both sides of my normal distribution curve, if I want to consider only one side obviously 0.05/2 that is way here it is 0.025.

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So you know how to use. Now I want to no change in the heart rate. What is the change? 42 is a change in the heart rate. So, I need to calculate the overall standard error understood. The overall standard error how do I do, both are same right 89.45 this is this variance of that 89.45 / 89.45 that comes out to be 178.9, I take square root of that I will get 13.4. Now I need to consider all the data sets. I have 1 set is 10, I have 9 degrees of freedom other set is 10, I have 9 degrees of freedom now I 18 degrees of freedom and I need to use 2 tail test, 2 tail table because we are talking about plus or minus here, you remember these equation right



. So, I use the both the regions p = 0.05 with 18 degrees of freedom, I read out this 2.101 I take 2.1 and 13.4 comes from here and 42 is the change in heart. The confidence limit for the change in heart at 95 % confidence is 14 to 70.

Do you understand the second part of the problem? The second part of the problem is you are suppose to find out the confidence limit for the change in the heart rate, change in the heart rate is originally it was 282 now it became 240. Now to find the confidence limit I use this equation if you remember this so, it is a



Now we have 89.45 for one set 89.45 for the second set, so total 178.9. I take a square root here, it becomes 13.4, that is the standard this whole portion. Now t I use 18 degrees of freedom do you understand why because, the first set has a 10 rats 9 degrees freedom, second has 10 rats 9 degrees of freedom totally 18 degrees of freedom. I used 2 tail because this is a \pm we are doing so and then t comes out form the table 0.05 18 degrees of freedom 2.101, so I substitute here I put 13.4. So when I do that I get the change in the heart rate, the confidence limit for the change in heart rate at 95 % confidence is 40 into 70. It is a interesting problem very very useful problem, we do quite a lot of these type of a studies in clinical trails we have a drug, you do give the drug and you see changes you know the original population and you are looking at change then you are trying to see whether the change is statistically significant or no. So this is varying.

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Let us look at another problem, the concentration of a drug in the brain of 6 rabbit's is determined after 5 minutes, after administrating the drug. You are injecting a drug on 6 rabbit's then after 5 minutes we are measuring their concentration, it comes out to be 40.2, 38.7, 41.6, 40.5, 43.2, 39.5. Now calculate the 99 % confidence interval for the true mean concentration that we can do, the mean concentration of the same drug in rats are reported to be 45 milligram per gram now this is the sample data from rabbit's these the

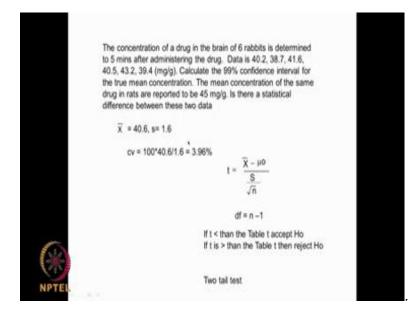
data which they got from rats is there a statistical significance difference between these 2 data. So 2 parts, 1 is calculating the 95, 99 % confidence is very simple. We can calculate

 \overline{X} from this data then a for 5 degrees of freedom with 95 %, 99 % confidence from the table we can calculate t and then we can calculate the standard error that is



that will give you my confidence limit right.

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Then the second part of the problem. Let us look at this average so, the average comes out to be 40.6 and S = 1.6. Of course, we can calculate the CV also in this case, but mainly we are more interested in this. So, we take it as a 40.6, we take this as 45 then you know the

S /6

and then degrees of freedom is 5, if t is < that the t calculated is less than the table t accept H_0 , t > table t reject H_0 ,. We are doing 2 tail test because, the question is, in rats it

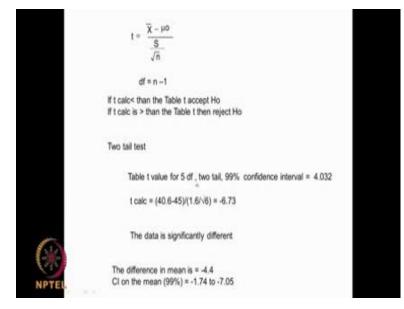
is 45 milligram per gram is there a statistical difference between these 2 data, is there are a statistical difference. The H_0 will be $\mu = \mu 0$, h alternate will be $\mu \neq \mu 0$.

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So we do that do that two tail test. For a 5 degrees of freedom 99 % confidence.

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Let us look at the table two tail test 99 % confidence means it is 0.01 for a 5 degrees of freedom you get this here 4.032. So this is the equation here,

$$X - \mu 0$$

, we do 40.6 - 45 and then you are standard deviation is 1.6 for the data. So, divided by

/6

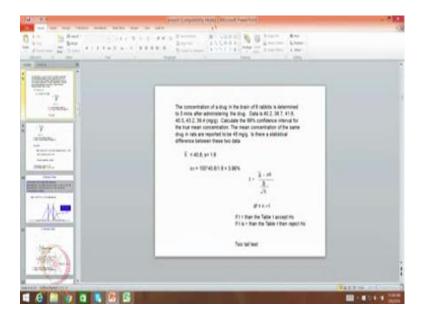
that will come to - 6.73 the data is significantly different. Data is significantly different so that means, we can reject the null hypothesis. That means we accept the alternative hypothesis. Now the difference in the mean 40.6 - 45 is 4.4 right. So confidence interval on the mean so obviously

t x s / square root n, t is 5 degrees of freedom. We get this



So we get a confidence interval on the mean as this actually, the difference in the mean is this, confidence interval on the mean is this. Now we can use the GraphPad also to do the same calculation, we can use not only the GraphPad we can also use our excel also to do the calculation. Shell we look at excel? Quite simple.

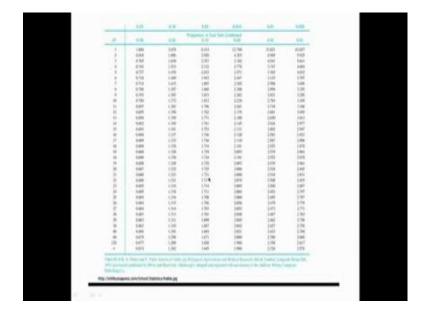
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Let us go back to excel, so the data is 40.2, 38.7, 41.6, 40.5, 43.2, 39.4 so we have a

sorry 38.7 and this data is not there. We have 6 data sets, now the command if you remember we go to this and there is something called a t-test. So when you look at the t-test what are the various things array 1. So we give the data set here and then we give the array 2. So here we give the 45, we give two tail test and then here we give pair or two sample t-test of course, as you can see we cannot do unfortunately we cannot do in excel this type of a calculations unfortunately. But we can use the other software here as you can see descriptive statistics, t-test here the GraphPad, here you have the 1 sample t-test. We can give the data here 40.2 then 38.7 then 41.6 and then 40.5, 43.2, 39.4 then we say we are comparing with 45. So, we have a 6 and then μ_0 is here so now when you say calculate now of course, this gives you only at a 95 % confidence interval. This is doing at the p value comes out to be 0.0011 and it is a statistically significant difference as you can see here. Even at 95 % confidence interval also it will come out to be statistically significant difference because if you look at the table here.

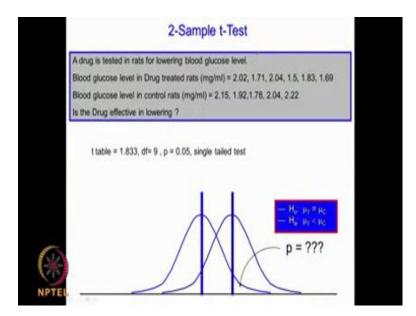
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Yeah if you look at the table here for a 5 degrees of freedom, for a 99 % confidence interval it is 4.032, for 95 % confidence interval it is 2.571. So your t value which we calculate is quite high here 6.6892 so obviously that number is very high, your t value which your calculating is pretty high so obviously, that number is much higher than the table t whether it is at 95 % or whether it is at 99 %. You see that with the excel we do not have way of calculating the 1 sample t-test, where as with GraphPad we can do this type of a calculation with is. Now let us go to next type of tests that is called a two

sample t-tests.

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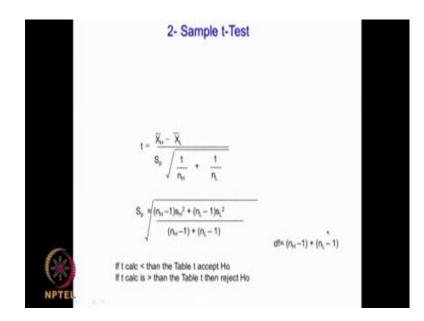


That means, I have 2 drugs I am comparing the performance of 2 drugs and I am trying tell whether there is a statistically significant difference or not or I am comparing a control and drug A which I have introducing in the market or I am comparing already existing drug and another drug or I am controlling, I am comparing the performance of a drug on a male population and a female population. We have 2 sets of samples, so you will have a x one bar and x two bar that is two means and then we are trying to see whether each of this sample means comes from the same population or they come from different population. For example, we are telling a drug is tested in rats for lowering blood glucose level this is drug treated rats, this is the glucose level and where as this is the controlled rats this is the glucose level. So we have 2 sets of samples, 6 samples for a drug treated rats, 6 for the controlled. Now is the drug effective in lowering the blood glucose level. For example imagine this sample comes from one population like this, imagine this sample comes from another population like this, now are these populations or overlapping so much that we can consider it as one single population or they are different that is a whole idea of this and is the drug effective in lowering that means, this is a single tailed test, for totally we have t value so we can find out and we can tell whether there is a statistically significant difference by saying whether the drug is lowering or there is no different. The null hypothesis will be

$$\mu_{\rm T}$$
 = $\mu_{\rm C}$

that means a test and control we say drug treated we call it test, c means control whereas alternate could be mu t less than mu c.

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This is the equation by which these things are calculated. Suppose, we have a number of data points for one set of sample, number of data points of another set of sample so the degrees of freedom will be this + this - 2, that is why in this particular problem so may have 3, 4, 5, 6; 3, 4, 5, 6 + 5 is 11, 11 - 2 is 9 degrees of freedom do you understand. So here, we have taken 6 data points for a drug treated here we have taken 5 data point. The advantage of 2 sample t-test I could have different sets of samples also in fact this particular equation if you look at this equation very carefully I can have any value of n h, I can have any value of n l so it did not be the same. So t value is given by this equation

$$\overline{X_{H}}$$
 – \overline{X}

that means, mean of one data set - mean of a another data set or sample set

$$\sqrt{\frac{1}{n_{H}}} + \frac{1}{n_{L}}$$

and then we have something called S_p here, this is the standard error. If you look it very carefully this for a one sample we used to have $S_p / square root of n$. This almost analogous to that here we have square root of $1 + n_H + 1 / 1 S_p$, but here s p it looks slightly different

$$S_{p} = (n_{H} - 1)s_{H}^{2} + (n_{L} - 1)s_{L}^{2}$$

$$(n_{H} - 1) + (n_{L} - 1)$$

the equation for S_p here. There are some similarities if you look at it this equation and the normal t equation for one sample t-test and so on. So, it is quite simple. We have 2 data sets I get the mean of this, I get the standard deviation of this $n_H = 6$, $n_L = to$ see 5. I substitute the standard deviations here square it here it will become 6 - 1, 5 - 1 so we here 1/6 1/5 and then here you can get the mean of both the data sets and you get the answer. So we can use this simple excel to calculate means and standard deviations and that is what I will do. That is the whole idea is we try to calculate t using fundas it is easy to use software's whether excel command like t-test it is easy to use the GraphPad like software or any commercial software, but then you need to know what is the underline equation that is very, very important and that is what I would like to emphasize on.

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1.71 1.92 2.04 1.78 1.5 2.04 1.83 2.22 1.69 Avg 1.798333 2.022 Std dev* 0.208367 0.176692 0.217083 0.12488 0.341963 0.194925 denomina 0.118033 t calc •1.89495			introl	
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Reject null hypothesis	Reject nul	I hypothesis		

With drug for the numbers are here, 6 numbers are here control that means you are not giving drug there are 5 numbers so we can get the average. Average is very simple you add all of them divided by 6. You add all of them divided by 5. Standard Deviation of this data set again you can use excel there is s t d e v function. This gives you the Standard Deviation of this, this gives you the standard deviation of this. Now you are squaring this also because I need to get here right, so I am squaring this and then I am adding this and then from there I calculate the t using this formula the denominator is 1/ 6 + 1 / 5 a square root here. So, I calculate this, from there I can calculate the t calculated so it is comes out to be - 1.89. Now t table is how many degrees of freedom 6 + 5 is 11. 11 - 2 is 9, so 9 degrees of freedom you go the t table it is a single tail 95 % because why single tail is the drug effective in lowering so it is a single tailed test. We go to the table it is a single tail 95 % 9 degrees of freedom it is comes to 1.833. The t calculated should be greater than 1.833 so it is comes to 1.89. So we can reject the null hypothesis. Please note it is only marginally higherd 1.89 and 1.833 is not very high. Where as if we take a 99 % confidence interval that is 0.01 for 9 degrees of freedom it is 2.82, t table is higher so we will not reject the null hypothesis. It is a very close problem, very close conclusion. I would not go with close conclusion I may do more experiments to be very sure you understand in this problem, with 95 % a 1.833 is a table so 1.89 so I can the reject the null hypothesis, with 99 % and a one tailed test you get much higher greater than 2.3 so you will not reject the null hypothesis and even 1.83 and 1.89 are very close.

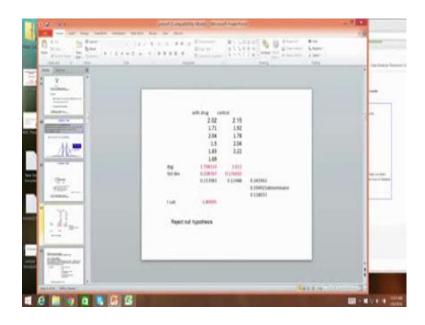
Although you can say I will reject the null hypothesis is a 95 % confidence, I would not go too much I may try to do more experiments so that we get a better difference between the t calculated and the t table do you understand. This is a very close call actually.

TTEST(array1,array2,1 Returns probability asso	tails,type) ociated with a Student's t-Test
	lata set. iber of distribution tails. If tails = 1, TTEST uses th f tails = 2, TTEST uses the two-tailed distribution.
If type equals	This test is performed
1	Paired
2	Two-sample equal variance (homoscedastic)
3	Two-sample unequal variance (heteroscedastic)

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The excel also can do this and I showed you last time just few minutes back excel cannot do one sample t-test, but it can do a two sample t-test, it can do a paired two sample t-test, it can do a two sample t-test when equal variance, two sample t-test when the unequal variance so the commands are array 1, array 2 tails and type. Array 1 is 1 first data set, array 2 is second data set, tails is one tail, 2 means two tail. So, from excel we get 0.043 that is the probability, so it is less. We can say we will reject the null hypothesis, but it is very close call. How do you do it in excel? We just put these data inside and then we do the calculations.

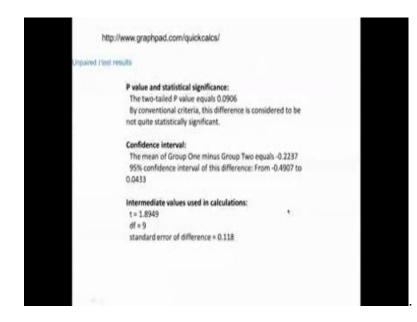
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We say 2.02, 1.71, 2.04, 1.5, 1.83, 1.69 then here 1.71, 2.04 then 2.15, 1.92, 1.78, 2.04 2.2 so use the same command t **test**. So we give the first data set and then comma we give the second data set then we comma it is a 1 sample t-test and we can give equal variance or unequal variance so if I put 3 .043. Basically I have given it as 3 that means unequal variance so I can even give it as equal. For unequal variance I am getting 0.43 yes the variances are unequal as you can see standard deviations are unequal here. It is giving 0.43 so we reject the null hypothesis hence, accept the alternate hypothesis. Same thing we can do with the, a GraphPad software also.

But you see it is going to be very close in GraphPad software. So what do we do? How do we do this in GraphPad? Let us go back there so here we have the t-test command compare 2 means. So we go there and then we continue.

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Because the data the t calculated and t table are very close to each other some software's may say it is not statistically significance, some software may say statistically significant. Especially you need to watch out with the data is very close to each other that is t calculated and t table is better to do more experiments to have more confidence in your conclusion. So we will continue on these t-tests in the next class also.

Thank you very much