Biostatistics and Design of Experiments
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Lecture – 11
t- Tests

Welcome to the course on Biostatistics and Design of Experiments. Today we will talk more about t- Test. We talked about one sample t- Test, where you have only one sample and comparing it with the global mean. Then we talked about two sample t- Test, where we are having two sets of samples and then trying to say whether these samples come from the same population or different population. For example, two different drugs you are comparing or two different activities we are comparing or two different instruments we are comparing. Now we will look at paired sample t- Test.

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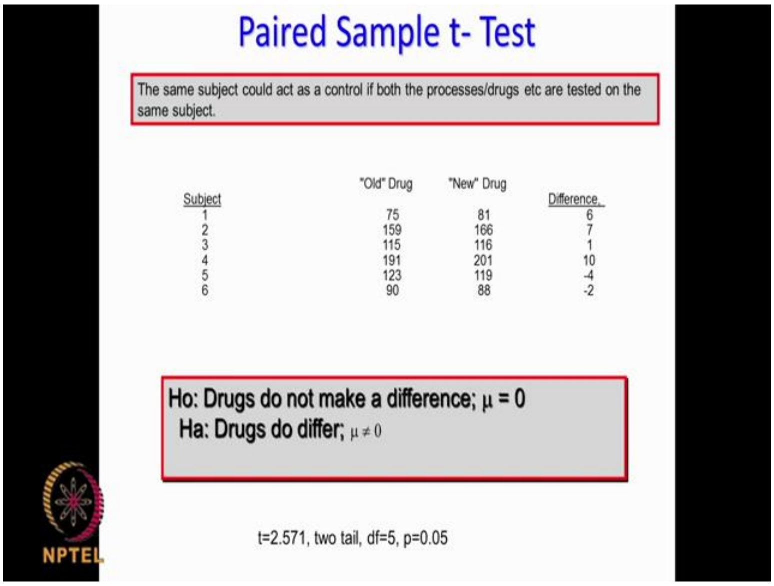

Paired sample t- Test is nothing but we use the same subject as control as well as the test. For example, I have 6 subjects because getting healthy volunteers, getting animals could be more difficult. Suppose I want to conduct a drug and a placebo comparison, I may need 6 animals for placebo, 6 animals for drug or if I want to test it on a human, then I may have 6 human volunteers for placebo, 6 for drugs, which is very expensive, time consuming it requires lots of resources. Sometimes what we do is, we make use of the same subjects, same animals both as the control as well as the test. In this particular example, I have 6 subjects, I am testing drug, old drug on subject 1 and old drug on subject 2 old drug on subject 3, 4, 5, 6 and I look at the performance. It could be a blood glucose lowering drug or it could be a heart beat lowering drug or cholesterol lowering drug. I wait for a few days so that assuming that affect of the old drug gets completely washed out and then I give this new drug on the same 6 volunteers.

The important point is, I should give sufficient times so that the effect of the old drug or old treatment or old (Refer Time: 02:24) gets completely washed out.

t=2.571, two tail, df=5, p=0.05

O	N	Diff
75	81	6
159	166	7
115	116	1
191	201	10
123	119	-4
90	88	-2
Avg		3
SD		5.51362
t		1.332

$$t = \frac{\bar{X} - \mu_0}{\frac{S}{\sqrt{n}}}$$

Accept null hypothesis

Then, I am testing the new drug and I am seeing again the effect on the same 6. If I subtract the effect of the old drug on volunteer 1 with the effect of the new drug on volunteer 1, I get something called the difference here. Theoretically, if the old drug and the new drug are the same the different should be equal to 0. But if, I see a statistically significant difference which is away from 0, then I can say the new drug is different from old drug or the new treatment is different from the old treatment and that is, what is paired sample t- Test. I take a difference and after that I will perform a one sample t-Test with the mu is equal to 0.

Let us take this example, 75 - 81 is 6, 159 - 166 is 7, 115 - 116 is 1, 191 - 201 is 10, 123 - 119. Actually, I am doing the other way 81 - 75 is 6, 166 - 159 is 7, 116 - 115 is 1, 201 - 191 is 10, 119 - 123 is - 4, 88 - 90 is - 2. Now, if I add all these, I will get some $\bar{X}$, I add all these I will get $\bar{X}$. I would like to tell whether this $\bar{X}$ is statistically different from the μ of 0. If my H_0 will be $\mu = 0$, H_a will be $\mu \neq 0$. If I want to show that new drug is different from the old drug, I can do a two tailed test because we are talking about only difference with the degree of freedom of 5 because we are looking at only this data now 6 data points. We are not looking at the whole lot such a two tailed test degrees of freedom is 5, $p = 0.05$. So $p = 0.05$ for a two tail test degrees of freedom is 5.

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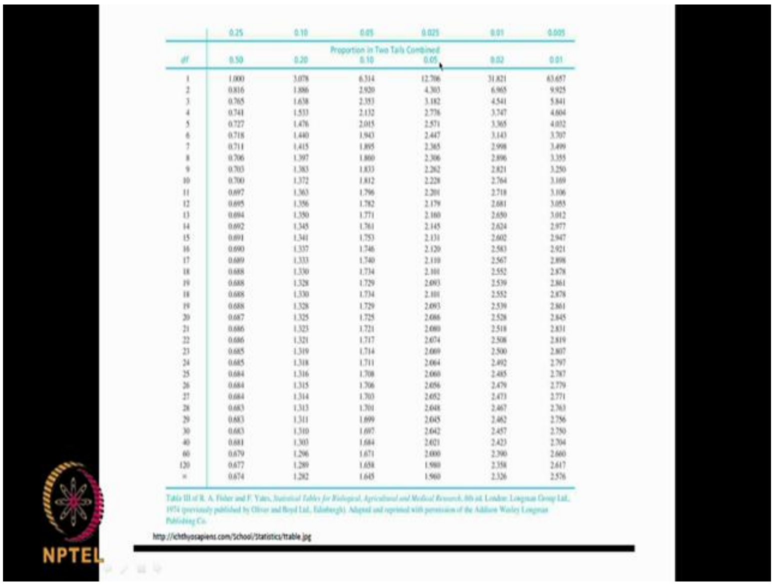

	0.25	0.10	0.05	0.025	0.01	0.005
			Proportion in Two Tails Combined			
df	0.50	0.20	0.10	0.05	0.02	0.01
1	1.000	3.078	6.314	12.706	31.821	63.657
2	0.816	1.886	2.920	4.303	6.965	9.925
3	0.765	1.638	2.353	3.182	4.541	5.841
4	0.741	1.533	2.132	2.776	3.747	4.604
5	0.727	1.476	2.015	2.571	3.365	4.032
6	0.718	1.440	1.943	2.447	3.143	3.707
7	0.711	1.415	1.895	2.365	2.998	3.499
8	0.706	1.397	1.860	2.306	2.896	3.355
9	0.703	1.383	1.833	2.262	2.821	3.250
10	0.700	1.372	1.812	2.228	2.764	3.169
11	0.697	1.363	1.796	2.201	2.718	3.106
12	0.695	1.356	1.782	2.179	2.681	3.055
13	0.694	1.350	1.771	2.160	2.650	3.012
14	0.692	1.345	1.761	2.145	2.624	2.977
15	0.691	1.341	1.753	2.131	2.602	2.947
16	0.690	1.337	1.746	2.120	2.583	2.921
17	0.689	1.333	1.740	2.110	2.567	2.898
18	0.688	1.330	1.734	2.101	2.552	2.878
19	0.688	1.328	1.729	2.093	2.539	2.861
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∞	0.674	1.282	1.645	1.960	2.326	2.576

Table III of R. A. Fisher and F. Yates, *Statistical Tables for Biological, Agricultural and Medical Research*, 6th ed. London: Longman Group Ltd., 1974 (previously published by Oliver and Boyd Ltd., Edinburgh). Adapted and reprinted with permission of the Addison Wesley Longman Publishing Co.

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My t value is 2.571 two tailed test remember that. So 6, 7, 1, 10 - 4 - 2, I calculate the $\bar{X}$ and I will try to see whether this $\bar{X}$ is statistically different from μ_0 of 0.

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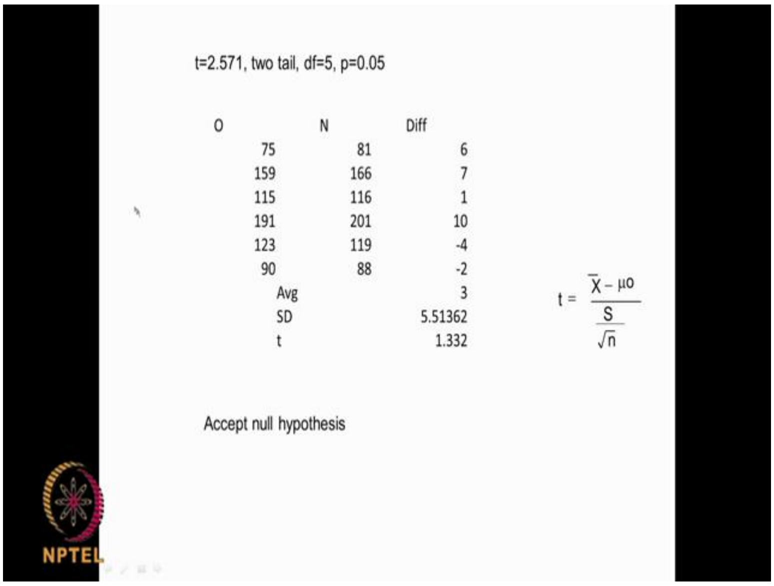

So 75 - 81; 6, 7, 1, 10, - 4, - 2, the average is 3. Does 3 come from a different population, where $\mu_0 = 0$, the standard deviation is 5.51. So t, I can calculate using this equation, remember this equation? $\bar{X}$ -, μ_0, / s, divided by square root of n; $\bar{X}$ is 3, μ_0 is 0, s is 5.51, square root of 6, so I get 1.332. Now t is 2.571, from the table assuming 5 degrees of freedom two tail p is equal to 0.05. There was no reason for you to reject the null hypothesis very straight forward, so this is the t 0.05. So 5 degrees of freedom we can use or for a two tailed test; the top 1 is one tailed test, the bottom 1 is two tailed test. So for 5 degrees of freedom 2.571 that is what it is.

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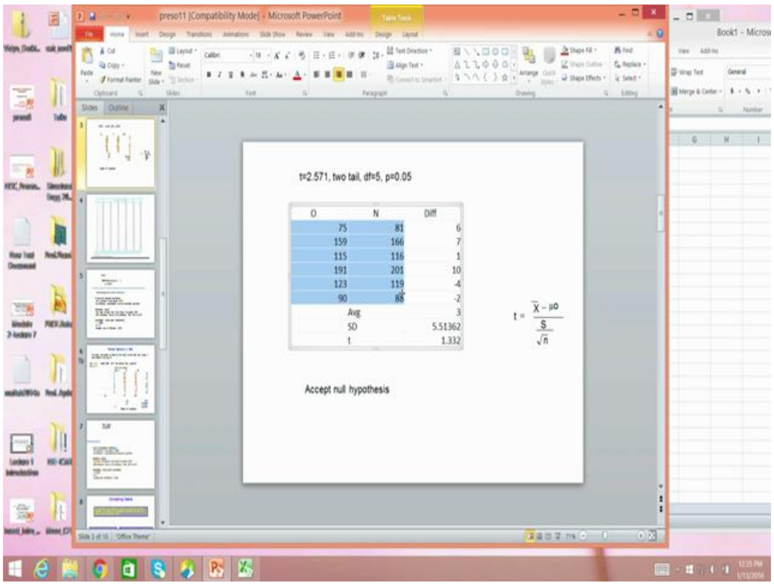

As GraphPad can do two sample t- Test. Here we do array 1 comma array 2 comma, I want to see difference. It is a two tailed test, it is the paired t- Test so we do 1 here, paired t- Test so 1 here. We get 0.24, so here we accept the null hypothesis. Do you understand this? So we use 1 for paired t- Test, 2 and 3, 4 for two sample t- Test. We can also do a two sample t- Test. I want you to see what is the result we get comma, comma tail is 2 comma we can say unequal variance 3, t value comes out very large. Even if you take it as equal variance, p value will come out to be large. That way paired t- Test is much better, as you can see to the paired t- Test is much better than a two sample t- Test. We can do it by the GraphPad software also, so graph pad software does a continuous distribution.

We have a paired t- Test concept here, enter the data here. We will go to data set copy 1 data set at a time, then we will again go to the excel, we copy another data set. You calculate now, it is not statistically significant here. So it is giving 0.91 as the p value, two tailed p value equals 0.91 that is here as it is given here two tailed p value. So here we are doing unpaired t- Test, whereas if you go and do a paired t- Test, calculate now by the difference is not considered. So it is giving 0.2401, same thing here we got. We got not significant here 2.20 and so on actually, t is 1.3328, 1.332. We can do it using the Excel function, which gives you both paired or unpaired two sample t- Test. But remember Excel cannot do a one sample t- Test, whereas a GraphPad can do. Then so three different approaches by which we can we seen and we can do this problem in that. Now let us look at another problem with the paired sample.

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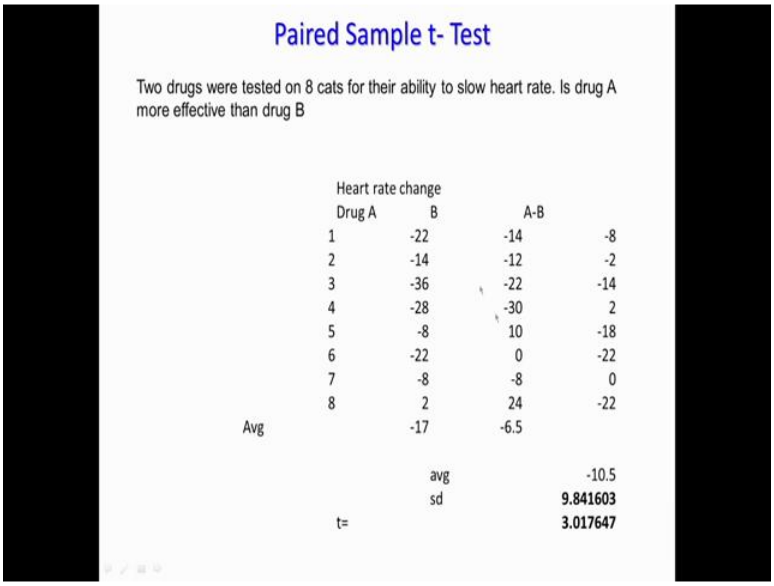

2 drugs were tested on 8 cats here. Drug 1, it is slowing the heart rate, so the heart rate has slowed, when I give drug a cat A, I mean cat 1 by - 22 that means, gone down 22. When I gave drug B, it went down - 14 and so on actually. So the average is - 17, average for this is - 6.5. Is drug A more effective than drug B? One way of doing this is we can assume this as two sample t- Test and do it or we can also do it by pair. Paired can be done only if you use the same subject for two different treatments. In this case drug A and drug B or placebo on drug or control and drug, same subject has to be used. I can do A minus B is minus 22 -14, - 14 - 12 and so on. I can add up overall that comes out to be minus 10.5, that gives you the mean, now these are the values. I can calculate

the t here $\mu_0 = 0$. I can say $\bar{X}$ is - 10.5 / 9.8 of then the square root of 8, that will come out be 3.017. I go to the table, H_0 μ is equal to 0, Ha $\mu < 0$, t table is 1.895 for 7 degrees of freedom.

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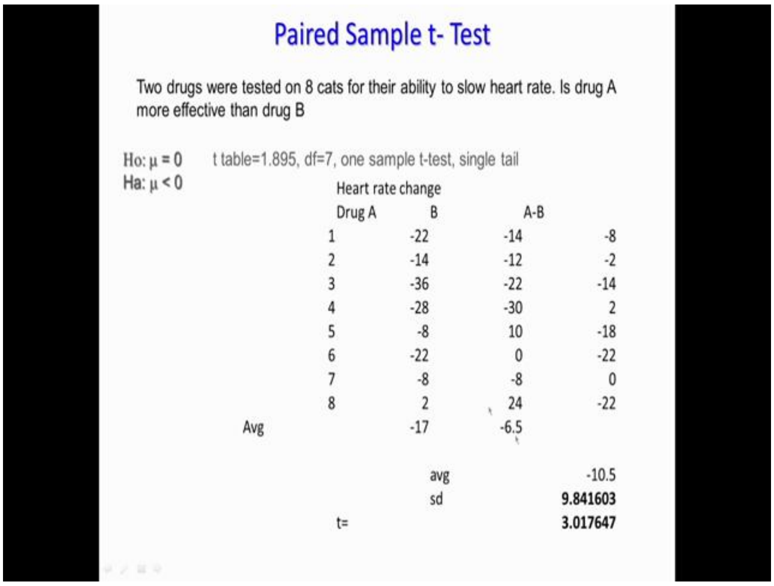

Let us go t table.

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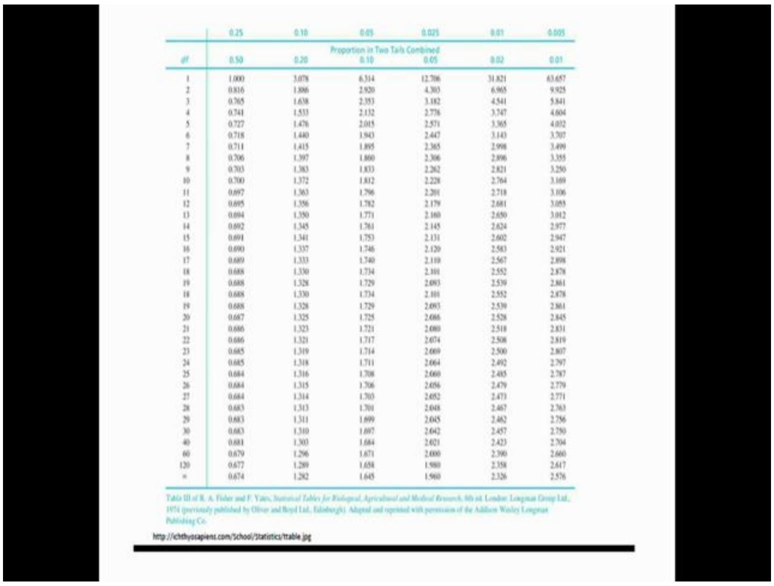

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For the top 1 corresponds to one tail test, bottom 1 corresponds to two tail test for 7 degrees of freedom 1.895. Remember for 95 you are using here 0.05 here so 1.895. 7

degrees of freedom table gives you for one sample test single tail 1.895, you are calculating as 3.017 so, what do you do?

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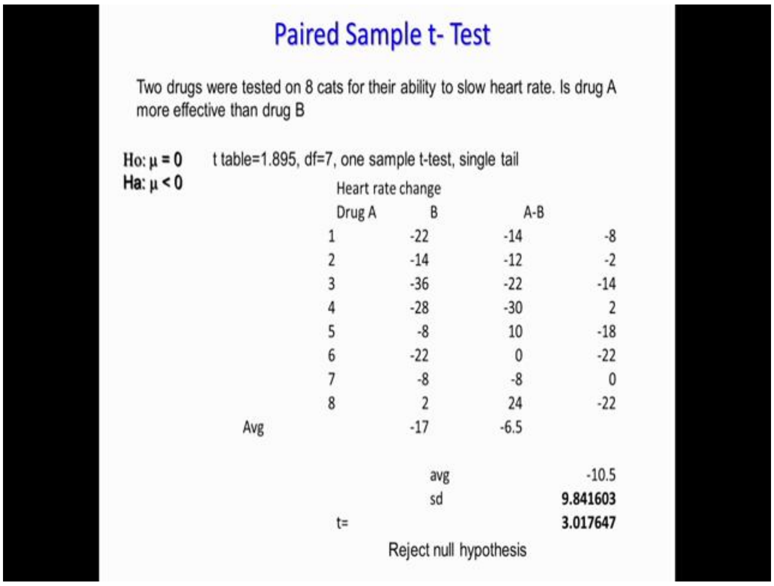

Reject null hypothesis, accept alternate. That means drug a lower is more effective than dug b that means, heart rate is less so understood? Here we will also do it by two sample t- Test we will do that now. But before that I want to show first you subtract a -b so it becomes only one sample and the μ = 0. If there is difference between the 2 drugs the mean should come out to be 0, if the mean is different, mean is now $\bar{X}$ of - 10.5, so what do I do?

$$t = \frac{\bar{X} - \mu_o}{\frac{S}{\sqrt{n}}}$$

; n is 10, so square root of 10; s is 9.8, $\bar{X}$ is - 10.5. When I calculate I get 3.01, table t is 1.895, 7 degrees of freedom, so for one sample t- Test single tail 1.895 so you reject the null hypothesis.

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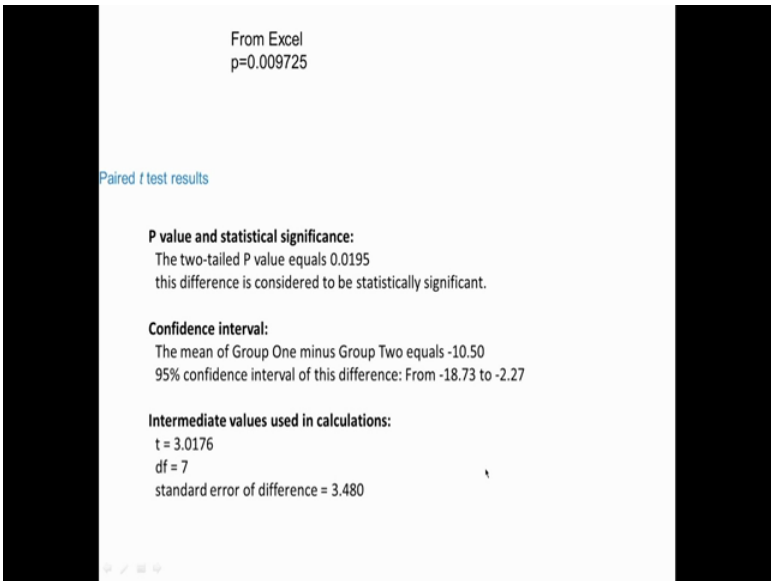

Let us do the same thing by using both Excel and the other software GraphPad.

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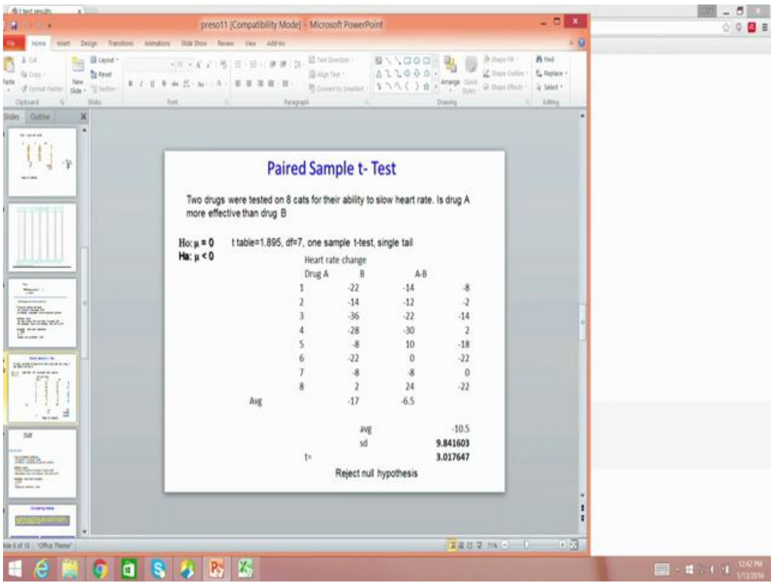

Let us go to Excel, first let me clear the whole thing. Excel cannot do a one Sample t-Test but it can do a paired t- Test. So I take these 2 data, control c, I am not interested in this. We will do t t s t, this data set comma this data set comma tail is 1 comma we are talking about paired t- Test so 0.009. As you can see here so we can say based on the paired sample study, we can reject the null hypothesis and accept the alternative hypothesis that means - 17 is much less in lowering, drug A is much better in lowering the heart rate when compared to drug B.

Let us do the same problem with two sample t- Test. So same command, I will do like this comma do like this comma again it is one tailed now 2 is equal variance to sample, 3 is unequal variance to sample so let me put 3. So it is come to 0.09, so this number is much larger than 0.05 that means we cannot reject the null hypothesis we are in trouble. So when I use a paired sample t- Test, I am able to see the real differences, I am rejecting the null hypothesis. When I use a two sample t- Test with unequal variance, I am not able to reject the null hypothesis. The differences are not being seen in two sample t- Test.

Here I am using equal variance, even here the p value in both these cases, p value is greater than 0.05. The null hypothesis cannot be rejected, whereas when I use the paired t- Test, I am rejecting the null hypothesis. Did you see that? So the paired t- Test is more accurate than two sample t- Test. But the point is you need to remember, I need to use the same subjects in both the cases. I am testing drug A and B on the same subject and then when I take a difference, I expect if there is no difference on the performance of the drug it should come out to be 0. So I will convert these 2 sets of sample into 1 set of sample by subtracting one from another. Then I will use the equation of

$\bar{X} - \mu_0$

μ_0 here will be 0 because I do not expect to see any difference divided by

$$\frac{S}{\sqrt{n}}$$

. That is what is a paired sample t- Test is, but the important point is when I treat the subjects with drug A, I give sufficient time and I assume that the effect of drug a is completely washed out. Then I give the drug B and then see the effect so there should not be any accumulation on the effect of drug a when I give the drug B then I will not be able to really decipher the 2 effect.

Here you saw that interestingly, when I do a two sample t- Test and even if you take the average for example, a v e r a g e comma, so this average is - 17 whereas, this average is

- 6.5, it looks very large but when I use a two sample t- Test because the variations are so large here. As you can see here the variation are so large that two sample t- Test gives you a probability of 0.09, which means that there is no statistically significant difference between these 2 data sets. Whereas when I perform paired t- Test, I am able to say there is statistically significant difference. Now we can use the GraphPad software also and do the same calculations here and I will take this.

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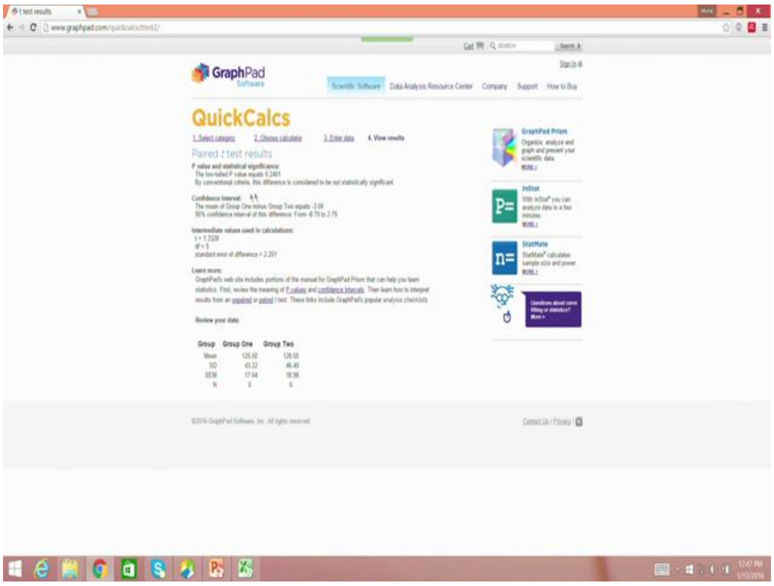

I will take this data and then I will say paired t- Test, calculate now, so it says by the difference is considered to be statistically significant. The p value comes out to be 0. 0195, this is a two tailed p value so for a one tail because in this problem we want to say drug A is more effective in lowering the heart beat.

For a one tail 0.0195 ÷ 2 is 0.009 and so on actually. When I use a paired sample t- Test, I am able to see the difference. Now let us look the unpaired t- Test, unpaired t- Test not statistically significant because my p value comes out to be 0.1859. So if I ÷2 because for a one tail I have to ÷ 2 it will come out to be 0.097, which is much larger than 0.05. So obviously, there p is not significant at 95 % so there is no reason for you to reject the null hypothesis.

So you noticed this paired sample t- Test is very powerful because it can see small, small differences unlike a two sample t- Test whether two sample t- Test equal variance or unequal variance. I showed you if I use Excel or if I use GraphPad when I run a two sample t- Test, the p value is much higher. So we will not be able to differentiate between these 2 drugs but whereas when we do a paired sample t- Test, where we are subtracting one sample from another sample to get the difference for each one of these catch. Then we get $\bar{X}$ here our null hypothesis will be μ_0 will = 0 but $\bar{X}$ is - 10.5. We want to know whether it comes from a different population, so obviously

$$\frac{\bar{X} - \mu o}{\frac{S}{\sqrt{n}}}$$

is what we try to do and we find that t value is much large 3.0 when compared to 1.9, 1.895 for one sample t- Test single tail 7 degree of freedom. Although two sample t- Test might not be able to give a good p value when we do a paired sample t- Test, we get a very good p value so we reject the null hypothesis.

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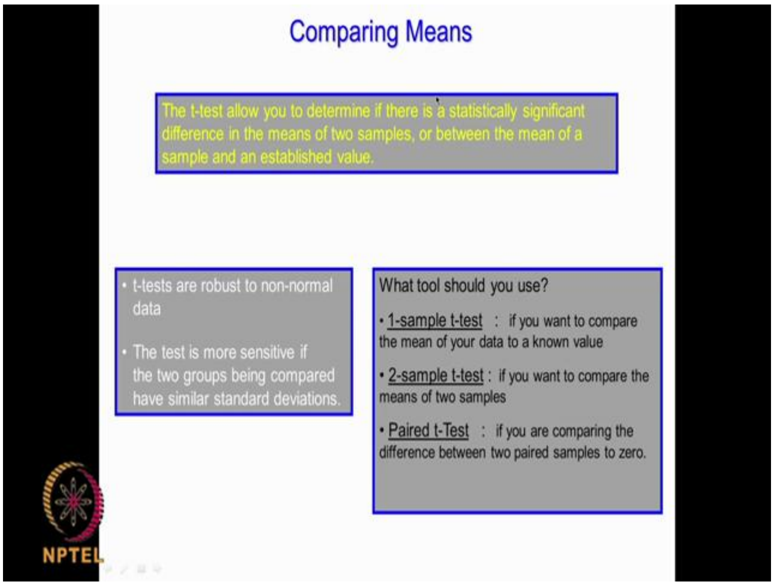

We looked at different types of t- Tests. All these t- Tests are meant for comparing means. So one sample t- Test that means we are comparing the mean of set a sample with a population mean. I take 10 students, I get their IQ and I say the compare it with the IQ of my university that is called a one sample t- Test. In Two sample t- Test we compare 2 sets of means, so I have a performance of a 1 student, I have a performance of another student then I am comparing these 2.

I am having a performance of drug A tested on say 10 rats or 10 human volunteers, I am comparing drug B tested on 20 rats or 20 human volunteers, I am trying to see whether they come from the same population or different population, that is called a two sample t- Test. Then we looked at paired t- Test, where I use the same subjects or same volunteers or same rats as for both control and test or drug A and drug B. So I subtract the performance because of drug A and because drug B. If there is no difference between drug A and B, I should get it as 0. Then I perform a one sample t- Test with μ_0 as 0. I use a

$$\frac{\bar{X} - \mu o}{\frac{S}{\sqrt{n}}}$$

So it becomes a one sample t- Test, I take a difference between these because it is paired and then I perform a one sample t- Test.

Paired t- Test is quite sensitive as I showed in one of the problems, whereas two sample t- Test is not able to differentiate between say drug A and drug B. When I do a paired t- Test, I am able to see a difference. But one important point you need to keep in mind is, when I test a same subjects with drug a and later with drug b, I have to be sure that the effect of drug a is completely washed and removed then I test the next drug otherwise you will start having combination effect or synergistic effect and so on actually.

You need to keep that point in mind, but in when you are doing clinical trails, you are taking subjects in different parts of a continent, different parts of cities, towns, so it is not possible for you to do a paired t- Test so you will have difference sets of samples. So you have to do a two sample t- Test, but one important point we also noticed is paired t- Test is able to give a very low p value. Whereas when we do the same analyze the same data set with two sample t- Test, the p value increases then we will not have the chance to reject the null hypothesis, so you should keep that in mind. All these t- Test are meant to compare means.

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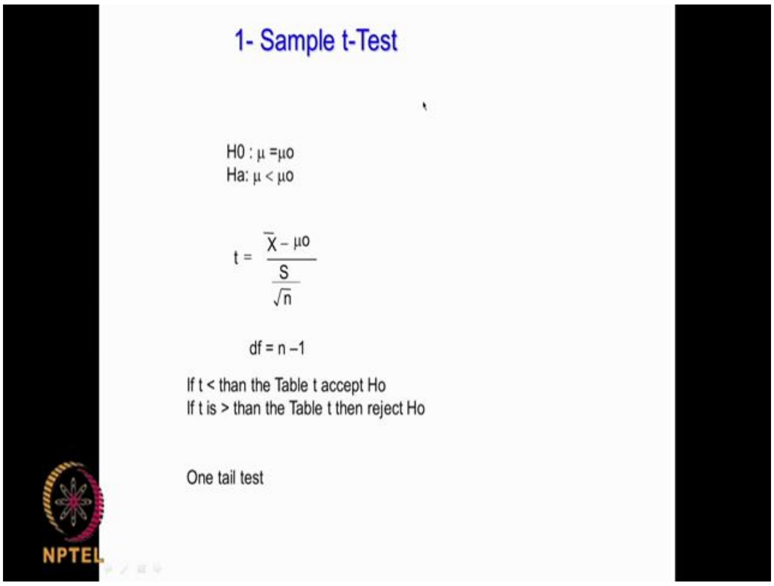

One sample t- Test to sum it up

$$\frac{\bar{X} - \mu o}{\frac{S}{\sqrt{n}}}$$

So we can use the same equation for paired t- Test also because we are converting two samples into one samples then the degrees of freedom is n - 1, then we get from the table some t value, then if the table t value and if the t calculated is less than the table we accept H_0. If t calculated is grater than the table we reject H_0.. This is one tailed test because we are talking about $\mu < \mu 0$. If μ is not equal to $\mu_0$then it becomes a two tailed test.

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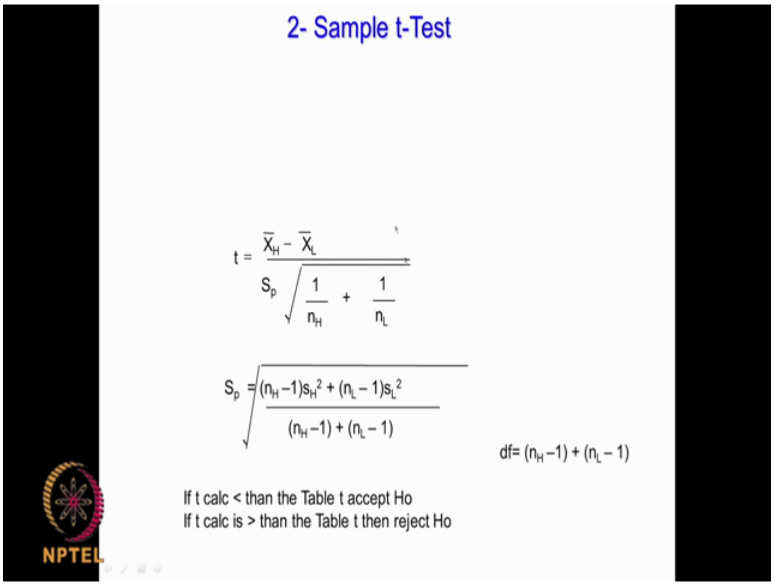

In two sample t- Test, this is the equation we have $\bar{X}_H$ is the mean of sample one, $\bar{X}_L$ is a mean of sample two and then we divide by standard errors,

$$\sqrt{\frac{1}{n_H} + \frac{1}{n_L}}$$

So, n_H is the number of data points for sample one, n_L is the number for data point for sample two. The degrees of freedom will be

$$df = (n_H - 1) + (n_L - 1)$$

like that. This is the equation which we use correct two sample t- Test. So let us in the next class talk about Tests that are involved in comparing variances or standard deviations.

Thank you very much for your time.