

Biostatistics and Design of Experiments
Prof. Mukesh Doble
Department of Biotechnology
Indian Institute of Technology, Madras

Lecture - 10
t- tests

We will continue with the course on Biostatistics and Design of Experiments. We have been talking about two sample t-tests in the previous class. Basically, two sample t-tests means we have two sets of samples, that means you are comparing drug a with drug b or you are comparing two different plants for the yields, so we take samples and then we want to find out whether those two samples come from the same population or they are from different population. Let us look at some more problems in that two-sample t-tests.

(Refer Slide Time: 00:41)

A sleep inducing drug was given to 30 subjects, so 30 subjects here and at the same time a placebo was given to 33 subjects we have it here actually and the time taken for them to get into the sleep was monitored. For example, if you look here we are given 15 minutes, 25 minutes, 30 minutes, that means it took some volunteers 15 minutes to sleep, when they took 2 volunteers when they took placebo and 0 volunteers when they took the drug, it took 4 volunteers who took the drug to sleep in 25 minutes and it took 3 volunteers to get into to sleep after 25 minutes so that is what these numbers are actually. So, these numbers are number of people who took so much minutes to sleep. Now the question is the with the drug lead to quicker on set of sleep that means, I want to know whether the average time taken to sleep by the drug people is less than the average time taken for the placebo group at 95 % confidence interval. It is quite simple, so we have 30 volunteers who took the drug and we have a 33 volunteer who took the placebo and this is the time so we can use the equation which I talked about in the previous class.

(Refer Slide Time: 02:14)

We need to calculate t,

$$t = \frac{\bar{X}_H - \bar{X}_L}{S_p \sqrt{\frac{1}{n_H} + \frac{1}{n_L}}}$$

, that means \bar{X}_H is your ave mean for a one group and this is the mean for another group, / by

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

that means n_H is number of samples for 1 group and n_L is number of samples for another group and then where S_p is given by $\frac{s_H^2 + s_L^2}{2}$ so S_H is the standard deviation for the first group, S_L is the standard deviation for the second group, and this is called the t calculated. If the t calculated is less than table t accept H_0 , if t calculated is greater than table t then reject H_0 . The degrees of freedom

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

so this in this particular problem it will be 63 - 2, 61 will be the degrees of freedom. What is the hypothesis? The null hypothesis H_0 will be μ_a is equal to μ_b that is the null hypothesis that means both the drug a could be the drug, b could be your placebo.

Now the alternate hypothesis could be μ_a less than μ_b , b could be your placebo, a could be your drug. So it is a two sample. 1 tail because we are talking about alternatives μ_a less than μ_b so it is a one tail or single tail, 95 % confidence interval, so we have to use this equation to calculate t and then compare it with the table t, if this t is less than the table t we accept H_0 , for 95 % confidence that is $p = 0.05$ one tail test, the t calculated here is $>$ table t then you reject the H_0 .

We can use an excel like software to calculate all these parameters the mean, the standard deviation of the individual samples all these things that is what we are going to do.

(Refer Slide Time: 04:21)

This is again I am showing you the problem here time taken to sleep 10 minutes, a number of subjects who took this drug, number of subjects who took the placebo. I need to calculate the average of each set, so how do I do, I will do 15×0 this, 15×2 is for the placebo, 25×4 is here, 25×3 is here, so like that I do go down, down, down and then I do all the summation here and then here I divide by 30 because there are 30 people who took the drug, then here I divide by 33 so it took 40.5 minutes or an average for the subjects who took drug to get into sleep. Whereas it took 43 minutes for subjects who took placebo.

Now the question is 43 and 40 appears to be different, but is 40.5 is statistically less than 43 at 95 % confidence interval. Now we need to calculate the standard deviation of each set, then we need to calculate this $n_H - 1$, that means here it could be $30 - 1$, $n_L - 1$ could be $33 - 1$ then standard deviation square that is variance take the square root substitute here and then calculate the t calculator and that is what I am doing in this part of the sheet. So, what do I do, I take an average this is my average is 40, then I will take the individual value and then multiplied by the then square it and then multiplied by the number of candidates. Here it will be $40 - 15^2 \times$ here 0, whereas for the other for the placebo I will do $43.03 - 15^2 \times 2$ like that I keep on doing down, down, down, down then I will do the summation then I will do the overall summation.

Then I can calculate the 251 is what I am getting here, 251 is what I am getting here and then I that is multiplied by $1 / 30 + 1 / 33$ that is this term here and then I am taking a square root here you can see here square root and then I am doing the difference is 2.5, because

$$\bar{X}_H - \bar{X}_L$$

you have so the difference is 2.5 and the denominator is 3.99, so t comes out to be 0.633. Now is this t statistically less than the table t or greater than the table t for 61 degrees of freedom for p 0.05, 1 tail test. Now I go to this it is a 1 tailed test 0.05, I go down 61, so it is 1.645. So obviously, this t calculated is much less. Then the t table so we cannot reject the null hypothesis, that means there is no statistically significant difference at 95 % confident limit between the placebo and the drug.

Although it looks, usually there is a decrease of 2.5 minutes on an average for a people who have taken the drug and compared to placebo to go to sleep but when you do this analysis we find that there is no statistically significant difference and 95 % confidence, there is no reason for you to reject the null hypothesis so you understand this problem. So, make use of this equation the equation is

$$\bar{X}_H - \bar{X}_L$$

that means mean of sample 1, mean of sample 2, if you call it this then n H is the number of items in that sample 1, these the number of items in the sample 2 and Sp is the standard error which you calculate using this formula, so these are the individual variances for this sample 1, the sample 2. The degrees of freedom here will be nH -nL - 2. Then you calculate t from this that is called t calculated then we use the table t and then if the t calculated is less than the table t, there is no reason for you to reject null hypothesis that means you have to accept null hypothesis. Only if the t calculated is greater than a table t then we reject the null hypothesis then we accept the alternate hypothesis.

In this particular case we want to know whether the drug reduces the onset of sleep. So, your hypothesis are null hypothesis is μ_a equal to μ_b , if I call a as my drug and my b as my placebo and the alternate hypothesis is μ_a is less than μ_b . How do you calculate each of these? is quite simple let me first talk about the averages.

How do you calculate averages, so what do you do? 15 x 0, 25 x 4 like that you keep on doing it then you sum it up and then divided by 30 that will give you the average time taken for the people who took the drug. For the placebo you do 15 x 2, 25 x 3, 30 x 3 and so on, again you add

it up and then $\div 33$ you get the average as 43.03 so you can call it this is \bar{X}_L , n_H will be 30, n_L will be 33. Now how do you calculate the variances here what do you do, if you want to look at the drug part of it so we say $40.5 - 15^2$ multiplied by the number here in this case 0, then $40.5 - 25^2$ multiplied by 4 people, $40.5 - 30^2$ multiplied by 3 \bar{X}_H that is \bar{X}_L what your writing here, then here $40.5 - 35^2 \times 6$ that is what you are writing.

So like that you keep on doing for the drug. And for the placebo what you do, you take a $43.03 - 15^2$ multiplied by 2 that is what comes here, $43.03 - 25^2$ multiplied 3 that is what it comes here and so on. And then you add up, you end up like this and then you can calculate these term, because you got this and then finally we end up with the $251 \div 1.1 / 30 + 1 / 33$ that is nothing but this ok because, 30 is the number of samples for the drug and 33 is the samples for placebo, then you are taking the square root that comes to 3.99 so that will cover the entire denominator part of it. The numerator is 2.5 right that is the difference in the averages, so you divide $2.5 / 3.99$ you will get 0.633 so that is the table t and according to sorry that that will be your calculated t and table t I have written as 1.96 but actually it should be.

(Refer Slide Time: 11:55)

If I take a 95 % or point p as 0.05 and I take 1 tail test I should be reading here so it should be 1.645 not 1.96, so this should read as 1.645. Whether it is 1.645 or 1.96 obviously, the table t is much higher, then your calculated t is obviously there is no reason for you to reject the null hypothesis at 95 % confidence interval. So, that means, the drug does not reduce the onset of a sleeping time when compared to the placebo. So, you understand how to do this problem very interesting we come across these types of problems quite a lot when you are performing clinical trials it could placebo, it could be drug b and that means another drug, so you may be comparing 2 drugs in the market and so on actually.

This table is very important, this top portion is related to the 1 tail, the bottom portion is related to 2 tail, so when we say 1 tail 0.05 you have to read this column, if you say 2 tail 0.05 you have to read this column please remember that. So, what is the relationship between 2 tail and 1 tail, 2 tail is totally is 0.05 so each side will be 0.025 so that is the relationship here understand.

(Refer Slide Time: 13:25)

Or we can use this is another table this gives you the outside portion here again you can see 0.025 relates to one tail. So, for two tail it is 0.05 so 1.96 is the t value.

(Refer Slide Time: 13:42)

Let us look at another problem another interesting problem. Artificial valve was placed in rats and mouse for 30 days; they were removed and tested for wear. This is very common when you are doing bio material design, when you are creating new material you keep it in a animal models and then see the mechanicals strength, changes in mechanical strength which could be tensile, compression, where, care and so on actually. Artificial valves where placed in rats and mouse for 30 days and they wear after 30 days were measured with the standard deviation so it is all given, it is not raw data but somebody has already done it and they have given it. Can it be said that the wear is less when the valves are placed in rats. This is again a 1 sample t-tests, because we are trying look at wear is less when the valves are placed in rats, so rats will be less than the mouse. So obviously, it is 1 sample t-tests, we can look at 95 or 99 depending upon the importance. The null hypothesis will be $\mu_{rats} = \mu_{mouse}$, the alternate hypothesis will be $\mu_{rats} < \mu_{mouse}$ that is what is (Refer Time: 15:03), say 99 or 95 or whatever you (Refer Time: 15:05), this is what I have said.

(Refer Slide Time: 15:05)

$H_0 \mu_{rat} = \mu_m$ mouse, $\mu_{rat} < \mu_m$. So, we are talking about 1 tail test, obviously, the degrees of freedom is 16, $10 + 8 - 2$ I said even if you remember. So, 16 degrees of freedom 1 tail test p is equal to 0.05 you go here, 16 degrees of freedom you can read out 1.746. Whatever calculation

we do, if the t value we get through calculation is greater than 1.746, then we can say we reject the null hypothesis, if the t value we calculate is less than 1.746, then there is no reason for you to reject the null hypothesis at 95 % confidence interval. So, we use the same equation if you remember

$$t = \frac{\bar{X}_H - \bar{X}_L}{S_p \sqrt{\frac{1}{n_H} + \frac{1}{n_L}}}$$

various p is given by right.

(Refer Slide Time: 16:06)

We can take rats here $n_R - 1$, so n_R for rats is 10, n_m for mouse is 8, the degrees of freedom is 16, $10 + 8 - 2$ then the standard deviation is given for rats, so squaring there standard deviation for mouse is given squaring that here we put 9, here we put $7 \div 9 + 7$ so we get S_p is equal to all these number.

(Refer Slide Time: 16:37)

Now, if you remember

$$t = \bar{X}_R - \bar{X}_m$$

that means average divided by S_p is whole thing which you have calculated here. Now X_R is given by this, X_m is given by this so divided by we are taking this then we are taking a

$\sqrt{(1/10+1/8)}$

we get 6.89. So obviously, the t value we calculate is much larger than the table t so we reject the null hypothesis at 95 % confidence interval, then we accept the alternate hypothesis. Please note there is a minus here, because I have taken rat minus X_m and the rat numbers are smaller. Even if you look at 99 % single tail test, let us look at 99 % single tail test that is you look here that is 0.1, 10 plus 8 minus 2 is 16.

So, let us look here 2.583, so 2.583 is still smaller than 6.89. So even at 99 % confidence interval we can reject the null hypothesis, whether it is 95 or 99 we can reject the null hypothesis that means you accept the alternate hypothesis. There is a statistically significant difference that means the wear is much more in rats than in mouse of the artificial wear.

So, you see you have done lot of these type of problems I am mean showing you quite a lot you can use simple excel and do all these calculations all you need to know is these set of equations here. You can use that t -test function that is available in excel, where when you have the raw data for the set of samples and another row data for another set of samples in the t -test function that is available in the excel you can do something called the two-sample t -test, when the variance are equal and the 2 sample t -test the variance are not equal.

But that t -test function cannot do this type of calculation because in this data, raw data is not given the average wear and standard deviation are already given whereas the excel function t -test can be done only when we have the raw data. So if we have data like this then obviously you have to use these equations remember that and you can we cannot use the graph pad online software also, if you have data like this you have to use these equations that is why I am spending lot of time on these equations that way you get an idea about the underlying mathematics behind calculating the t . Otherwise, softwares can blindly give you some t and then it can tell you it is statistically not significant or statistically significant so anybody can do that, but you need to know what are the underlying equations that are used when you calculate 2

sample t-tests or 1 sample t-tests or confidence interval that is why I am spending lot of time on these equations.

And for example, these types of problem we cannot do it with excel you have to do it manually like this you have to take these equation substitute here and then using a calculator or something do the calculations. Whereas a excel or even graph pad can do if you are given raw data. What does that raw data means? For example if you are talking about 10 rats, I will have all the 10 wear value know 0.0045, 0.0048 like that and if I have 8 mouse I will have all the 8 wear values. Whereas here the average and standard deviation of this 10-data set is given that means somebody has already done that calculation and given you. So, the excels or graph pad will not be able to handle this type of data so you need to use these equations.

So, we talked about 2 sample t-tests where you can compare 2 different sets of samples and I told you how to calculate the t using this equation and you say whether the equation t is greater than the t the table for a one tail or a two-tail test, if it is a greater than the table we reject the null hypothesis, if it is less than the t table we accept the null hypothesis. And also, we looked at one tail test where we are saying that the drug induces sleep much faster than placebo, they were in the valve when they are implanted in rats is much more and so on actually. So, in that sort of thing we are talking about one tail test. I also showed you the table how to use the table and that is very, very important, that table it tells you the p values for one tail on the top and the p values for a two tail here and this one gives you the degrees of freedom. In a two-sample t-test please remember the degrees of freedom will be number of samples for a sample set 1 and number of samples for a sample set 2 minus 2 remember that. Whereas when you are doing a one sample t-test it will be total number of samples minus 1, that will be the number of degrees of freedom. So, we will continue and we will talk about paired t-tests in the next class.

Thank you very much for your time