2019 – 2020 Dr. Fazıl Küçük Faculty of Medicine, EMU Year 1 Committee 3

Biostatistics Course

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Covered Lectures in Y1C1

- What is statistics and biostatistics?
- Statistics in medical research
- Designing research (Study types)
- Types of data
- Describing data with graphics
- Describing data with numbers

Covered Lectures in Y1C2

- What is probability and probability distribution?
- Bayes' Theorem
- Principles of statistical analysis
- Elements of statistical inference
- Introduction to statistical analysis
- Sampling, distribution and estimation
- Testing statistical hypothesis
- Types of errors in statistical inference
- Difference between parametric and nonparametric methods; Introduction to parametric methods
- One sample t-test, unpaired t-test and paired t-test

Topics of Y1C3 biostatistics course

- Review of statistical inference table and introduction to nonparametric methods
- Sign test, Mann-Whitney U test, Wilcoxon test (paired sample)
- Chi-square test of independence
- Chi-square test of homogeneity
- Comparing risk: Relative Risk and Odds ratio
- Some basic concepts for diagnosis tests
- Correlation
- Simple Regression
- Approach to Evidence-based Medicine
- Statistical packages
- Interpretation of computer outputs
- Systematic reviews and Meta analysis

Statistics

Biostatistics

Design of a research study

Study types Types of data

Sample size determination

Sampling methods

Concept of: Evidence based medicine Fundamental concepts

Probability

Bayes'
theorem
and
screening
tests

Probability distributions: Discrete / Continuous

Analysis of data

Descriptive analysis

Graphics/ Tables

Numbers e.g. Mean, Std. Dev, etc. Inferential analysis

Estimation

Hypothesis testing

Types of hypothesis tests

Risk comparison

Correlation

Regression

Lecture 1 of Y1C3

Review of statistical inference table and introduction to nonparametric methods Sign test, Mann-Whitney U test, Wilcoxon test (paired sample)

Review of Statistical Inference

Statistical Inference

Estimation

- Point Estimate
- Interval Estimate

Hypothesis Testing

- Parametric Methods
- Nonparametric Methods

Recall Steps in Hypothesis Testing

- 1. Evaluate data
- 2. Review assumptions
 - parameters related to the population, distribution
- 3. State hypotheses
- 4A. Select and calculate test statistics
- ▶ 4B. Find p-value
- 5. State decision rule
- ▶ 6. Make statistical decision:
 - Reject H_0 (conclude H_1 is true)
 - Fail to reject H_0 (conclude H_0 might be true)
- 7. Conclusion

Parametric vs. Nonparametric test procedures

Parametric

- Assume that data are normally distributed
- Require interval scale or ratio scale
- More complex computations
- Tend to have less power when there are extreme values (outliers)
- Make inferences about mean(s)

Nonparametric

- There is no assumption on the distribution of the data
- Data measured on any scale
- Simpler computations
- Can be useful for dealing with extreme values (outliers)
- Make inferences about median(s)

How to determine which hypothesis test to use?

- When deciding on the appropriate hypothesis test, we should consider:
 - 1) The type of the data
 - The distribution of the data
 - 3) The number of groups of observations
 - 4) If there are groups, the independence/dependence of the group of observations

1 sample vs hypothesized value comparison

Comparisons

More than 2

Comparisons

Crosstabulation

Correlation Coefficient (This measures the degree and determines the direction of linear co-relation

between two coninous variables, x and y)

samples

Quantitative

Data

Categorical

(qualitative) Data

distributed)&populations have and having small sample size equal variances (homogenity of variances) One-sample t-test

Non parametric Methods

No assumption. It is preferred

when normallity is violated

(n < 30)

Signed rank test or

Wilcoxon Rank Sum Test

Mann-Whitney U Test

Wilcoxon Matched Pairs Test

Kruskal-Wallis Test

If p<0.05 use Dunn Multiple

Comparison Test

Friedman Test

If p<0.05 use Dunn Multiple

Comparison Test

X2-Test * Homogenity test *Independence test If the association is significant; Phi or Cramer's V coefficients can be used

İn order to measure the degree and to

determine the direction of the

association.

McNemar X² test (2x2) or Stuart-Maxwell testi(3x3)

Spearman Correlation Coefficient

Parametric Methods Assumption: Data have to be

sampled from Gaussian

distribution (normally

Unpaired t-test

Paired t-test

One way-ANOVA Test If p<0.05 according to F-test,

Multiple Comparison Tests are needed;

such as; Tukey, Bonferroni, Scheffé Multiple

Comparison Tests

To compare all vs. only with control group,

Dunnett Multiple Comparison Test can be

used. **Repeated Measures ANOVA Test**

If p<0.05 according to F-test, Multiple

Comparison Tests are neededStudent such

as Newman-Keuls

Pearson Correlation Coefficient

Independent Samples 2 samples

Paired Samples or Matched

Pair Samples

Independent Samples

Paired Samples or Matched

Pair Samples

Independent Samples

Paired Samples or

Matched Pair Samples

Recall t-tests

1) One sample t-test

- We are interested in whether the mean of the variable in the population of interest differ from a specific hypothesized mean.
- Comparison parameter has been estimated from prior research or is derived from theory.

2) Independent samples (or Unpaired) t-test

We compare the means of two independent populations.

3) Paired samples t-test

We compare two dependent groups.

A researcher is planning a psychological intervention study, but before he proceeds he wants to characterize his participants' depression levels. He tests each participant on a particular depression index, where anyone who achieves a score of 4.0 is deemed to have 'normal' levels of depression. Lower scores indicate less depression and higher scores indicate greater depression. He has recruited 40 participants to take part in the study. Depression scores are recorded in the variable dep_score. He wants to know whether his sample is representative of the normal population (i.e., do they score statistically significantly differently from 4.0).

Data: 3.68, 3.98, 3.72, 3.98, ..., 4.26, 3.51, 4.04 (n=40)

- The example is a one sample design to compare the population mean with a known threshold.
- Can we directly apply one-sample t-test?
 - No!
 - First, we need to check if the assumption of normality is met.

Normality check

- In order to check if the data is normally distributed or not, Shapiro-Wilk test is conducted.
 - H_0 : Data is normally distributed
 - H_1 : Data is not normally distributed

Tests of Normality

	Koln	nogorov-Smir	nov ^a	Shapiro-Wilk_		
	Statistic	df	Sig.	Statistic df S		
dep_score	.103	40	.200*	.958	40	.138

^{*.} This is a lower bound of the true significance.

Lilliefors Significance Correction

P-value=0.138 > 0.05We fail to reject H_0

Data is normally distributed

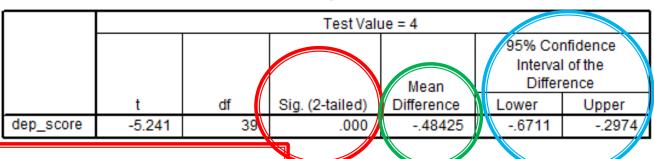
- Since data is normally distributed, one sample t-test can be conducted:
 - H_0 : The mean depression score of the study population is not different than 4 ($\mu_{dep_score} = 4$)
 - H_1 : The mean depression score of the study population is different than 4 ($\mu_{dep\ score} \neq 4$)

Results:

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
dep_score	40	3.5158	.58434	.09239

One-Sample Test



P-value=0.000 < 0.05We reject H_0

 $\mu_{dep_score} \neq 4$, it is less than 4

Lower by 0.48

CI of the difference is (-0.67,-0.3)

How to report these results?

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
dep_score	40	3.5158	.58434	.09239

One-Sample Test

		Test Value = 4							
					95% Coi	nfidence			
					Interval of the				
				Mean	Difference				
	t	df	Sig. (2-tailed)	Difference	Lower	Upper			
dep_score	-5.241	39	.000	48425	6711	2974			

Mean depression score (3.52 \pm 0.58) was lower than the normal depression score of 4.0, with a statistically significant difference of 0.48 (95% CI, 0.3 to 0.67),

$$t(39) = -5.241, p < 0.0005.$$

Example: Independent Samples t-test

The table below shows the cholesterol levels of 20 patients from City A, and 18 patients from City B. Are the means of cholesterol levels from these two cities statistically different from each other?

	1	2						
city A	4.1	5.3	4.9	 5.2	6.4	5.1	5.8	(n=20)
city B	6.6	5.2	5.9	 6.3	6.4			(n=18)

Example: Independent Samples t-test

- The example is a two independent samples design to compare the population means.
- Can we directly apply independent samples ttest?
 - No!
 - First, we need to check the assumptions
 - Observations in each group should follow a normal distribution
 - Shapiro-Wilk test
 - The variances in the two samples should be equal (homogeneous)
 - Levene's test for homogeneity of variances (this test is done within the procedure of t-test / so no extra work needed)

Normality check

- In order to check if the data in groups is normally distributed or not, Shapiro-Wilk test is conducted.
 - H_0 : Data is normally distributed
 - H_1 : Data is not normally distributed

Tests of Normality

		Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	group	Statistic	df	Sig.	Statistic	df	Sig.
Cholesterol (mmol/l)	City A	.117	20	.200*	.973	20	.815
	City B	.165	18	.200*	.932	18	.215

^{*.} This is a lower bound of the true significance.

Lilliefors Significance Correction

For City A: p-value=0.815 > 0.05

We fail to reject H_0 ; data from City A is normal

For City B: p-value=0.215 > 0.05

We fail to reject H_0 ; data from City B is normal

Example: Independent Samples t-test

- Since data is normally distributed, independent samples t-test can be conducted:
 - H_0 : The mean cholesterol levels in City A is not different than the mean cholesterol level in City B $(\mu_{City\ A} = \mu_{City\ B})$
 - H_1 : The mean cholesterol levels in City A is different than the mean cholesterol level in City B $(\mu_{City\ A} \neq \mu_{City\ B})$

Example: Independent Samples t-test

Results:

Group Statistics

	group	N	Mean	Std. Deviation	Std. Error Mean
Cholesterol (mmol/l)	City A	20	5.670	.7740	.1731
	City B	18	6.006	.6958	.1640

Independent Samples Test

Levene's Test for Equality of Variances				t-test for Equality of Means						
							Mean	Std. Error	95% Cor Interval Differ	l of the
		F	Sig.	t	df	Sig. (2-tailed)	Difference	Difference	Lower	Upper
Cholesterol (mmol/l)	Equal variances assumed	.522	.475	-1.399	36	.170	3356	.2398	8219	.1508
	Equal variances not assumed			-1.407	36.000	.168	3356	.2384	8191	.1480

Independent Samples Test

		Levene's Equality of	Test for Variances			t-test fo	r Equality of Me	eans		
	Mean Std. Erro		Std. Error Difference	95% Confidence Interval of the Difference Lower Upper						
Cholesterol (mmol/l)	Equal variances assumed	.522	.475	-1.399	36	.170	3356	.2398	8219	.1508
	Equal variances not assumed			-1.407	36.000	.168	3356	.2384	8191	.1480

$$H_0: \sigma_1^2 = \sigma_2^2$$

$$H_1$$
: $\sigma_1^2 \neq \sigma_2^2$

$$p = 0.475 > 0.05$$

Fail to reject H_0 Variances are equal

$$H_0$$
: $\mu_{City\ A} = \mu_{City\ B}$

$$H_1$$
: $\mu_{City A} \neq \mu_{City B}$

$$p = 0.17 > 0.05$$

Fail to reject H_0 Means are not different

Mean cholesterol level in City A(5.67 \pm 0.77) does not significantly differ from the mean cholesterol level in City B (6 \pm 0.7), t(36) = -1.399, p = 0.17.

Example: Paired Samples t-test

In a tumor size study, two doctors were shown the same set of tumor pictures. The volume of tumor was measured (in cm³) by two separate physicians under similar conditions, and the following values are obtained. Is there a difference in tumor volume measurement based on physician?

Tumor	Dr1	Dr2
1	15.8	17.2
2	22.3	20.3
3	14.5	14.2
4	15.7	18.5
5	26.8	28.0
6	24.0	24.8
7	21.8	20.3
8	23.0	25.4
9	29.3	27.5
10	20.5	19.7

Example: Paired Samples t-test

- The example is a paired samples design to compare the population means
- Can we directly apply paired samples t-test?
 - No!
 - First, we need to check the assumption of normality:
 - The differences should be normally distributed
 - Shapiro-Wilk test

Normality check

- In order to check if the data is normally distributed or not, Shapiro-Wilk test is conducted.
 - H_0 : Data is normally distributed
 - H_1 : Data is not normally distributed

Tests of Normality

	Koln	nogorov-Smir	nov ^a	Shapiro-Wilk		
	Statistic	df	Sig.	Statistic df Sig.		
difference	.138	10	.200*	.930	10	.452

^{*.} This is a lower bound of the true significance.

Lilliefors Significance Correction

P-value=0.452 > 0.05We fail to reject H_0

Data is normally distributed

Example: Paired Samples t-test

- Since data is normally distributed, paired samples t-test can be conducted:
 - H_0 : There is no difference in tumor volume measurement based on physician $(\mu_{dr1} = \mu_{dr2})$
 - H_1 : There is a difference in tumor volume measurement based on physician $(\mu_{dr1} \neq \mu_{dr2})$

Example: Paired Samples t-test

Results:

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair	dr1	21.3700	10	4.87762	1.54244
1	dr2	21.5900	10	4.60880	1.45743

High positive correlation

(r = 0.934, p < 0.0005),

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	dr1 & dr2	10	.934	.000

Paired Samples Test

		Paired Differences							
				Std. Error	Interva	95% Confidence Interval of the Difference			
		Mean	Std. Deviation	Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	dr1 - dr2	22000	1.74407	.55152	-1.46763	1.02763	399	9	.699

Paired Samples Test

		Paired Differences							
				Std. Error	95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	dr1 - dr2	22000	1.74407	.55152	-1.46763	1.02763	399	9	.699

We can conclude that mean volume measurement by physician 121.37 ± 4.88 is not significantly different than the mean volume measurement by physician 21.59 ± 4.61 ; t(9) = -0.399; p = 0.699

$$H_0: \mu_{dr1} = \mu_{dr2}$$

 $H_1: \mu_{dr1} \neq \mu_{dr2}$

$$p = 0.699 > 0.05$$

Fail to reject H_0 Means are not different

Nonparametric Tests for Quantitative Data

What if data is not normally distributed and ttests cannot be conducted?

Type of Design	Parametric Test	Nonparametric Test
One sample	One-sample t-test	Sign Test
Two indepent samples	Independent- samples t-test	Mann Whitney U test
Two paired sample	Paired-samples t- test	Wilcoxon Signed Ranks test
	-	
	•	
	-	•

One Sample Case

Example: For the 21 patients with medullary carcinoma, percentages of cells with aberrations were calculated. Suppose that, in general population, the mean percentage of choromosomal aberrations has been reliably estimated to be 3.90. Does the population of patients with medullary carcinoma differ from the general population in the percentage of cells with choromosomal aberrations?

Subject	Percentages of cells with aberrations	Subject	Percentages of cells with aberrations
1	13.9	12	5.0
2	10.9	13	2.0
3	9.3	14	2.0
4	5.2	15	17.1
5	14.0	16	13.4
6	2.0	17	6.0
7	3.2	18	14.0
8	12.0	19	18.0
9	13.0	20	13.6
10	11.3	21	5.4
11	12.2		

Example: Doctor Z et al. reported the endurance scores of 12 animals during a 48-hour session of discrimination responding. Conduct a test to see whether the investigators may conclude at the 0.05 level of significance that the median endurance score of animals with electrodes implanted in the forebrain is equal to 97.5 or not.

Subject	Lead Level
1	98.1
2	82.4
3	97.7
3 4	84.4
	97.8
5 6	94.5
7 8	81.7
	97.5
9 10	80.3
	94.6
11 12	85.5
12	82.6

One Sample Case

Example:

- 21 patients
- the mean percentage in general population is estimated to be 3.90.
- Does the population of patients with medullary carcinoma differ from the general population by means of the percentage of cells with choromosomal aberrations?

Shapiro-Wilk Test of Normality -> p=0.057

Parametric: One sample t-test

Hypotheses

 H_0 : $\mu = 3.90$ H_1 : $\mu \neq 3.90$

Sample mean = 9.69 Sample Std Dev = 5.14 Mean Difference = 5.79 p<0.0005; Reject H₀

Conclusion: Mean percentage of cells with chromosomal aberrations in the population of patients with MC is significantly different (higher) than the general population.

Example:

- the endurance scores of 12 animals during a 48-hour session of discrimination responding.
- test whether the investigators may conclude that the median endurance score of animals with electrodes implanted in the forebrain is equal to 97.5 or not.

Shapiro-Wilk Test of Normality -> p= 0.013

Nonparametric: One sample signed rank test

Hypotheses

H₀: The median in the population equals 97.5.

 H_1 : The median in the population does not equal 97.5.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The median of lead_level equals 97.500.	One-Sample Wilcoxon Signed Rank Test	.016	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Conclusion: The median endurance score of animals with electrodes implanted in the forebrain significantly differs from 97.5

Two Independent Samples

Example: The following data from Schechter et al. [1973] deal with sodium chloride preference as related to hypertension. Two groups, 12 normal and 10 hypertensive subjects, were isolated for a week and compared with respect to Na+ intake. Data includes the average daily Na+ intakes (in milligrams). Compare the average daily Na+ intake of the hypertensive subjects with that of the normal volunteers by means of an appropriate hypothesis test.

Average daily Na+ intakes							
Subjects	Hypertensive Group (n=10)	Control Group (n=12)					
1	92.8	39.7					
2	54.8	24.1					
3	51.6	66.3					
4	61.7	22.5					
5	52.4	47.1					
6	84.5	42.2					
7	42.1	39.3					
8	62.2	43.6					
9	43.7	51.3					
10	55.6	44.3					
11		43.1					
12		39.8					

Example: A company is interested in marketing a clotting agent that reduces blood loss when an accident causes an internal injury such as liver trauma. The company conducts an experiment in which a controlled liver injury is induced in pigs and blood loss is measured. Pigs are randomized as to whether they receive the drug after the injury or do not receive drug therapy—the treatment and control groups, respectively. The data were taken from a study in which there were 10 pigs in the treatment group and 10 in the control group. The blood loss was measured in milliliters. We want to conclude whether blood loss in two groups differs or not.

	Blood Loss in mililiters								
Subjects	Treatment Group (n=10)	Control Group (n=10)							
1	786	543							
2	375	666							
3	4446	455							
4	2886	823							
5	478	1716							
6	587	797							
7	434	2828							
8	4764	1251							
9	3281	702							
10	3837	1078							

Na+ intake

Two Independent Samples

Blood loss

Shapiro-Wilk Test of Normality

Te sts of Normality

		Kolr	nogorov-S mii	nov ^a		Shapiro-Wilk	
	group	Statistic	df	Sig.	Statistic	df	Sig.
daily Na+ intakes	hypertensive	.250	10	.076	.864	10	.085
	control	.242	12	.051	.904	12	.180

a. Lilliefors Significance Correction

Parametric: Independent samples t-test

Hypotheses

 H_0 : $\mu_1 = \mu_2$ H_1 : $\mu_1 \neq \mu_2$

Group Statistics

	group	N	Mean	Std. Deviation	Std. Error Mean
daily Na+ intakes	hypertensive	10	60.140	16.4804	5.2116
	control	12	41.942	11.4023	3.2916

Independent Samples Test

		Levene's Equality of	Test for Variances	s t-test for Equality of I			r Equality of M	leans		
							Mean	Std. Error	95% Co Interval Differ	of the
		F	Sig.	t	df	Sig. (2-tailed)	Difference	Difference	Lower	Upper
daily Na+ intakes	Equal variances assumed	1.403	.250	3.054	20	.006	18.1983	5.9596	5.7668	30.6299
	Equal variances not assumed			2.952	15.583	.010	18.1983	6.1640	5.1028	31.2939

p=0.006; Reject H_0

Conclusion: There is a significant difference between two groups by means of their daily Na+ intake.

Daily Na+ intake in hypertensive patients is higher than the daily Na+ intake in control group.

Shapiro-Wilk Test of Normality

	Tests of N	ormality			
Kolr	nogorov-S mi	rnov ^a	9	Shapiro-Wilk	

a. Lilliefors Significance Correction

Nonparametric: Mann Whitney U test

.097

Hypotheses

H₀: There is no difference between treatment and control groups by means of the median of blood loss. H₁: There is a difference between treatment and control groups by means of the median of blood loss.

Test Statistics^b

	blood_loss
Mann-Whitney U	43.000
Wilcoxon W	98.000
Z	529
Asymp. Sig. (2-tailed)	.597
Exact Sig. [2*(1-tailed Sig.)]	.631 ^a

- a. Not corrected for ties.
- b. Grouping Variable: group

p=0.597; Fail to reject H_0

Conclusion: There is no significant difference between treatment and control groups with respect to their blood loss.

Paired Samples

Example: A random sample of 10 young men was taken and the heart rate (HR) of each young man was measured before and after having a cup of caffeinated coffee. The results were given (beats / min). Does caffeinated coffee have any effect on the heart rate of young men?

Heart	Heart rates (beats per minute)					
Subjects	HR before coffee	HR after coffee				
1	68	74				
2	64	68				
3	52	60				
4	76	72				
5	78	76				
6	62	68				
7	66	72				
8	76	76				
9	78	80				
10	60	64				

Example: Weis and Peak studied the effects of oxytocin on blood pressure during anesthesia. The subjects were 11 women, 19 to 31 years of age and weighted 103 to 251 pounds and were in the first trimester of pregnancy. They had been anesthetized for dilation and curettage, and were injected with 0.1 unit/kg of oxytocin. The arterial blood pressures before and after oxytocin are recorded. Can we conclude on the basis of these data that oxytocin lowers the arterial blood pressure of women?

Arterial E	Arterial Blood Pressures (mmHg)					
Subjects	Before	After				
1	95	92				
2	173	90				
3	94	80				
4	97	59				
5	81	72				
6	100	46				
7	97	75				
8	104	92				
9	72	70				
10	101	34				
11	83	78				

Paired Samples

Shapiro-Wilk Test of Normality

Tests of Normality

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
difference	.201	10	.200*	.924	10	.394

Parametric: Paired t-test

Hypotheses

H₀: The mean heart rate of young man does not differ after having a cup of caffeinated coffee.

 $(\mu_{HR_before} = \mu_{HR_after})$

H₁: The mean heart rate of young man differs after having a cup of caffeinated coffee.

 $(\mu_{HR_before} \neq \mu_{HR_after})$

Paired Samples Statistics

					Std. Error
		Mean	N	Std. Deviation	Mean
Pair	HRbefore	68.00	10	8.844	2.797
1	HRafter	71.00	10	6.055	1.915

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	HRbefore & HRafter	10	.929	.000

Paired Samples Test

			•					
	Paired Differences							
			Std. Error	95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1 HRbefore - HRafter	-3.000	3.916	1.238	-5.801	199	-2.423	9	.038

p=0.038; Reject H_0

Conclusion: The mean heart rate significantly increases after coffee.

Shapiro-Wilk Test of Normality

Tests of Normality

	Shapiro-Wilk		
	Statistic	df	Sig.
ABPafter_ABPbefore	.851	11	.044

Nonparametric: Wilcoxon matched pairs test

Hypotheses

H₀: The median of Arterial Blood Pressure does not differ after oxytocin is applied.

H₁: The median of Arterial Blood Pressure differs after oxytocin is applied.

Test Statisticsb

	ABPafter -
	ABPbefore
Z	-2.934 ^a
Asymp. Sig. (2-tailed)	.003

Statistics

		ABPbefore	ABPafter
Ν	Valid	11	11
	Missing	0	0
Medi	an	97.00	75.00

- a. Based on positive ranks.
- b. Wilcoxon Signed Ranks Test

p=0.003; Reject H_0

Conclusion: There is a significant decrease in median blood pressure of pregnant women after oxytocin is applied.