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The Effect of Liquid Nitrogen Administration on the Number of Chondrocytes in Male *Rattus norvegicus* of Wistar Strain

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ABSTRACT

Introduction: This study investigates the impact of liquid nitrogen administration on histology and chondrocyte count in experimental animal cartilage, highlighting the limited benefits of cryosurgery on cartilage, a common treatment in orthopedics and skeletal muscle injuries.
Methods: The research design in this study was a random sampling experimental test on white male rats (Rattus norvegicus). Experimental animals that met the inclusion criteria were randomly divided into four groups: the control group and three groups that received different durations of liquid nitrogen administration (one, five, and ten minutes). Furthermore, an evaluation of the histology and the number of chondrocytes of the experimental animals was carried out and analyzed by statistical tests using SPSS 26.0.
Results: The One-way ANOVA analysis showed a significant difference in chondrocytes in the liquid nitrogen group compared to the control group. Furthermore, the results of the Tukey test showed that giving liquid nitrogen for 5 minutes and 10 minutes significantly reduced the number of chondrocytes in experimental animals. There was a negative correlation between liquid nitrogen administration and chondrocytes duration.
Discussion: Mechanical destruction of cells, including chondrocytes, occurs because the extreme temperature differences of liquid nitrogen can freeze the cells, becoming an ice crystal that can impair the physical structure of cells and membranes.
Conclusion: Liquid nitrogen administration significantly impacts chondrocyte levels in Rattus norvegicus strain cartilage, with duration negatively correlated with chondrocyte levels, suggesting a decrease with longer administration.

Keywords: cryosurgery, chondrocytes, neoplasm

INTRODUCTION

Primary bone tumors are neoplasms that are relatively rare compared to all types of neoplasms found in humans. Sarcomas in bones are around 0.2% of the neoplasm population, where soft tissue sarcomas are found ten times more often than soft tissue sarcomas. The incidence of bone sarcoma in America and Europe is 0.8 per 100,000 population and 1% of the incidence of all malignant tumors, and it occurs more often in children. The three most common primary bone tumors are osteosarcoma, Ewing's sarcoma, and chondrosarcoma. The incidence of chondrosarcoma is around 35% of all malignant bone tumors.[1]

Management of malignant bone tumors is currently still a problem for orthopedic surgeons in Indonesia, especially with limited resources such as diagnostic facilities, therapy, and oncology teams. Around 70-80% of all malignant tumors can be treated with limb salvage surgery, and this is currently a very beneficial therapeutic option for patients where this procedure has functional benefits for patients of productive age. Complications were found to be higher in patients who underwent limb-salvage surgery compared to amputation, with the consequence that further surgery was necessary for the patient. [1] Limb-salvage surgery is currently the standard treatment for malignant bone tumors, including osteosarcoma; megaprosthesis is generally used in reconstructive surgery. In addition, liquid nitrogen is developing as a local adjuvant for deep curettage. [2,3]

Cryosurgery is a therapeutic modality that uses extreme cold temperatures from liquid nitrogen to destroy neoplasm cells. One of the techniques used in orthopedics aims to remove neoplastic tissue from bones by immersing the pathological bone in liquid nitrogen. Liquid nitrogen eliminates pathological tissue through cell apoptosis caused by the formation of ice crystals in cells and failure of microcirculation. In this process, the tissue around the bone immersed in a liquid nitrogen solution will be exposed to the freezing effect of nitrogen, which will affect the structure of the surrounding tissue, one of which is chondrocytes. Liquid nitrogen has become one of the procedures that is more often chosen due to its advantages and benefits. Many studies have proven that liquid nitrogen has excellent joint preservation results with a very low recurrence rate. The use of procedures with liquid nitrogen is also less invasive. It has a very good level of effectiveness, making this procedure a safe, easy, cheap, but still effective option in managing neoplasms in the bone. [4,5]

The treatment of bone tumors has developed rapidly in the last three decades. This technique is called Cryosurgery, which has been proven to achieve excellent local control of various benign-aggressive and malignant bone tumors. It is easy to obtain, and liquid nitrogen is cheaper. [4]

MATERIALS & METHODS

Research Design

The research design was a randomized control trial experimental study on male white rats (Rattus norvegicus). Objective: To determine the comparison of the increase in the number of chondrocyte activities in white rats (Wistar Rattus Norvegicus strain) that were exposed to liquid nitrogen and those that were not exposed to liquid nitrogen

Research Samples

The samples used were male Wistar rats, adults aged ± 6 months and weighing 500 grams. Determination of sample size for experimental research with a completely randomized design using the Federer formula.[6]

This study used mice that met the inclusion and exclusion criteria and underwent adaptation (acclimatization) for seven days, then randomly divided into four groups:

1. Negative control group: normal mice without liquid nitrogen administration
2. Treatment group 1: mice were immersed in liquid nitrogen for 1 minute, and then samples were taken
3. Treatment group 2: mice were immersed in liquid nitrogen for 5 minutes, and then samples were taken
4. Treatment group 3: mice were immersed in liquid nitrogen for 10 minutes, and then samples were taken

Liquid nitrogen

Liquid nitrogen is a cryogenic liquid that is colorless, odorless, noncorrosive, and nonflammable. It is currently used in bone sterilization methods.

Chondrocytes

Chondrocytes are working cells that form cartilage and unite to increase the mineralization of new bone matrix. Histologically, active osteoblasts appear as large, cuboidal cells on the surface of bones.

The cells are accountable for the production of cartilage and are needed for the process of endochondral ossification, which is crucial for the growth and development of bones. Chondrocytes have a significant function in healing bone fractures by imitating the process of skeletal growth. The examination was conducted under a microscope with 400x magnification after the preparation stage with H&E staining.[7]

Rattus novergicus wistar strain

The type of experimental animal, Rattus norvegicus Wistar strain, follows the inclusion criteria: White Rats are around 3-4 months old, male, weigh 180-200 grams, and are physically healthy (active movements, not dull fur, respond to the surrounding environment. The sex of the experimental animal is male. The selection of experimental animals that are all male is based on the following considerations:

a. Using experimental animals that are all male can avoid bias caused by the effects of the estrogen cycle, which can influence research results.
b. Using male experimental animals is intended to make the sample more homogeneous. The mice's body weight was determined by weighing them.

Data Analysis

The acquired data is examined and presented in tables. Subsequently, the sample is assessed for normalcy and homogeneity. If the power is homogeneous and normally distributed, proceed using the One-way ANOVA difference analysis test and the Pearson correlation test using SPSS 26.

Histological Examination and Chondrocyte Count

After taking rat tibia bone tissue from the knee joint to the ankle joint to make it easier to identify the fracture position, the tissue was then taken to the Anatomical Pathology Laboratory, Faculty of Medicine, Brawijaya University, Malang to make preparations and check the number of chondrocytes.

1. The tissue taken is immersed in media containing 10% formaldehyde.
2. Then the tissue is first decalcified with 5% nitric acid solution for ± 1 week, waiting until the tissue is soft and can be cut into small pieces
3. Carry out the dehydration process using alcohol:
   - 70% alcohol for 1 hour
   - 80% alcohol for 1 hour
   - 90% alcohol for 1 hour
   - 95% alcohol for 1 hour
   - 99% alcohol for 1 hour
   - 100% alcohol for 1 hour
4. The cleaning process involves immersing the dehydrated material in a xylol solution for two 30-minute intervals.
5. The process of block construction, known as embedding, is performed.
6. Mount the block onto the rotary microtome and create thin longitudinal cuts with a thickness ranging from 3 to 5 µ.
7. Subsequently, the incision results are immersed in a water bath to facilitate appropriate expansion. They are then allocated to an identified glass item.
8. The coloring process involved using Hematoxylin and eosin (HE), and a cover glass was placed on top.
9. Chondrocyte observations were conducted by quantitatively counting the number of cells in 10 tiny fields of view using an Olympus BX-51 dot Slide microscope equipped with an Olympus XC10 camera set at 400x magnification. Next, the calculation results are examined and evaluated.

RESULT

Data from the analysis of chondrocyte results obtained from four treatment groups, starting from the negative control, nitrogen administration for 1 minute to 10 minutes, will be used to carry out statistical calculations using descriptive techniques and inference using SPSS 26.0. Descriptive analysis aims to offer a comprehensive portrayal of the variables being studied.

This descriptive study aims to provide a comprehensive picture of the current status of patient data. Refer to Table 1 for a summary of each variable.

Table 1. Descriptive Analysis

Group	N	Mean ± sd	Minimum	Maximum
Negative Control	9	12.22 ± 1.56	10	15
1 Minute Liquid Nitrogen Administration	9	10.00 ± 1.66	8	13
5 Minute Liquid Nitrogen Administration	9	7.56 ± 2.13	4	11
10 Minute Liquid Nitrogen Administration	9	4.22 ± 1.64	2	7

According to the given description, this is a broad overview of the data acquired from the study findings, which has not yet demonstrated the actual conclusions of the investigation. To determine the research findings, a hypothesis test will be conducted using the One-way ANOVA test with a significance level of 5% or a confidence level of 95% for chondrocyte parameters. Before employing parametric statistics, it is necessary to assess the normality and homogeneity of the data.

Normality Test

This test is used to ascertain the normality of the data distribution. If the data from the test results follows a normal distribution, then one of the requirements for doing parametric statistical analysis has been met. The test technique is conducted utilizing the Kolmogorov-Smirnov test under the specified conditions:

Hypothesis used:

H_0: data is normally distributed

H_1: data is not normally distributed

If the sig value (p-value) > 0.05, H_0 is accepted, which means normality is met.[8] The normality test results are in Table 2.

Table 2. Normality Test

	Chondrocyte
N	36
P	0.746

The normality test findings indicate that the significant value (p) for chondrocytes, as determined by the Kolmogorov-Smirnov test, is 0.746. Since the p-value is greater than 0.05, the null hypothesis (H0) is accepted, indicating that the chondrocyte data follows a normal distribution.

Homogeneity Test

Before testing using One-way ANOVA, the data obtained for each treatment were analyzed for data homogeneity using the homogeneity of variance test (Levene test) to determine whether the data used had the same variance.[9] The homogeneity test results can be seen in Table 3.

Table 3. Homogeneity Test

	Levene Statistic	Df1	Df2	p
Chondrocyte	0.282	3	32	0.838

The test findings indicate that the chondrocyte homogeneity test has a p-value of 0.838, greater than the significance level of 0.05 (alpha). Given that the p-value is greater than 0.05, the null hypothesis (Ho) is accepted. Therefore, it can be inferred that the analyzed data has a uniform distribution. One-way ANOVA is applicable for testing chondrocytes.

One-way ANOVA Test

Analysis of Variance (ANOVA) is a statistical test used to compare the means of three or more groups. Specifically, the One-way ANOVA test is used when only one independent variable exists. It helps determine if an analysis was conducted utilizing the One-way ANOVA Test to ascertain if there are significant distinctions between each treatment and between one treatment and another. [10] The test results are displayed in Table 4.

Table 4. One-way ANOVA Test

Parameter	F count	p	Interpretation
Chondrocyte	34,112	0.0000	Significant

Based on the results of the One-way ANOVA analysis in the table above, it was

found that the p-value for chondrocytes was 0.000. The result of the p-value <0.05 for the chondrocyte parameters was to reject Ho, meaning there was a significant difference in chondrocytes in the liquid nitrogen administration group with an error rate of 5%.

Tukey's test

After the One-way ANOVA test was carried out, the comparison results between each group were then continued using the Tukey test. Tukey test results with differences in group average values are shown if they have a p-value <0.05.[10] The Tukey test results for each parameter are seen in Table 5.

Table 5. Tukey's Test

Group Comparison		Mean Difference	p	Interpretation
K1	K2	2.222	0.054	Not Significant
	K3	4.667	0.000	Significant
	K4	8.0000	0.000	Significant
K2	K3	2.444	0.029	Significant
	K4	5.778	0.000	Significant
K3	K4	3.333	0.002	Significant

The results of the Tukey test for chondrocytes showed that the negative control group had significantly different chondrocytes when given liquid nitrogen for 5 minutes and liquid nitrogen for 10 minutes. However, the negative control still had an insignificant difference in chondrocytes when given liquid nitrogen for 1 minute. The comparison results of giving liquid nitrogen for 1 minute had significantly different chondrocytes from giving liquid nitrogen for 1 minute and giving liquid nitrogen for 10 minutes. Likewise, giving liquid nitrogen for 5 minutes also had significantly different chondrocytes given liquid nitrogen for 10 minutes.

Relationship between Duration of Liquid Nitrogen Administration and Chondrocytes

The correlation test is used to determine whether there is a relationship; it can be seen from the significance value, and the relationship's strength can be seen from the correlation coefficient value or r. The correlation value (r) ranges from 1 to -1; a value closer to 1 or -1 means the relationship between two variables is getting stronger. Conversely, a value approaching 0 means the relationship between two variables is weaker. Positive values indicate a unidirectional relationship (X increases, then Y increases), and negative values indicate an inverse relationship (X increases, then Y decreases).[11] Correlation categories can be divided according to the table 6 below.

Table 6. Guidelines for Providing Interpretation of Correlation Coefficients [11]

Size of Correlation	Interpretation
0.90 to 1.00 (−0.90 to −1.00)	Very high positive (negative) correlation
0.70 to 0.90 (−0.70 to −0.90)	High positive (negative) correlation
0.50 to 0.70 (−0.50 to −0.70)	Moderate positive (negative) correlation
0.30 to 0.50 (−0.30 to −0.50)	Low positive (negative) correlation
0.00 to 0.30 (0.00 to −0.30)	Negligible correlation

The Pearson correlation test was used to calculate the relationship between the duration of therapy and chondrocytes because it has a ratio data scale and is normally distributed. The results of the person correlation test can be seen in Table 7.

Table 7. Pearson Correlation Test

Variable	r	p	Interpretation
Duration of Liquid Nitrogen Administration – Chondrocytes	-0.858	0.000	Significant

Based on the results of Table 7, the relationship between the duration of liquid nitrogen administration - chondrocytes is known to have a correlation value of -0.858 with a p-value of 0.000, a correlation value of 0.858 indicates that the duration of liquid nitrogen administration and chondrocytes has a relationship in a very high category, the negative direction of the relationship suggests that a decrease will follow the more prolonged liquid nitrogen administration in chondrocytes. Meanwhile, with a p-value = 0.000 < 0.05 (α = 5%), it can be concluded that there is a significant relationship between the duration of administration of liquid nitrogen and chondrocytes.

DISCUSSION

Although ordinary light microscopes may seem less sophisticated in cellular studies in the 21st century, using light microscopes to study chondrocytes and articular cartilage has been around for at least 100 years. The light microscope is known to be capable of being used to examine all aspects of the musculoskeletal system, including the cartilage and chondrocytes within the cartilage, the curved structure of collagen fibers throughout the tissue, the pattern of split lines on the joint surface, and the consequences of external loading on the morphology of the extracellular matrix (ECM) in cartilage.[12] This supports using a light microscope in this research to study and evaluate chondrocyte cells from each existing treatment, which is adequate and good.

Table 1 shows a general description of the variables of this study, where this study consisted of 4 groups, each consisting of 9 mice, bringing the total number of mice tested in this study to 36. From this study it was found that the negative control group had the highest average chondrocytes (mean ± sd as 12.22 ± 1.56) with the highest maximum number of chondrocytes also a maximum of 15, a minimum of 10, which was then followed by the group given liquid nitrogen for 1 minute (mean ± sd 10.00 ± 1.66, maximum 13, minimum 8), the group given liquid nitrogen for 5 minutes (mean ± sd 7.56 ± 2.13, maximum 11, minimum 4). Finally, the group was given liquid nitrogen for 10 minutes (mean ± sd 4.22 ± 1.64, maximum 7, minimum).

From this general overview data, it can be concluded at a glance that there is a decrease in the number of chondrocytes, which is consistent with the administration of liquid nitrogen compared to the negative control group.

The results of this research can occur because when living cells are exposed to liquid nitrogen, the rapid cooling process causes the water in and around the cells to freeze into ice crystals. These ice crystals can form inside or outside cells, so they can cause physical damage to cell structures and membranes. Ice crystals can cause mechanical damage to cell walls, resulting in cell rupture, and can also cause an osmotic decrease by cell drying. In addition, sudden temperature changes can cause rapid changes in cell pressure, further damaging cellular structures and resulting in cell death. Cold temperatures can also slow or stop important metabolic processes necessary for cells to function properly, resulting in further damage and cell death.[13,14]

Cartilage tissue already lacks blood vessels and has a limited supply of nutrients, making it more susceptible to damage from extreme temperature changes. Damage caused by exposure to liquid nitrogen can disrupt the production and maintenance of the extracellular matrix, resulting in cartilage tissue degradation and potential joint problems.[15,16]

Statistical analysis of data on differences in the number of chondrocytes with liquid

nitrogen administration supports the hypothesis of this study, where there is a change in the number of chondrocytes after freezing treatment with liquid nitrogen based on treatment time. Based on the One-way ANOVA test analysis results, it was found that the p-value for chondrocytes was 0.000. The results of the p-value <0.05 for chondrocyte parameters indicate significant differences in chondrocytes in the liquid nitrogen administration group with an error rate of 5%.

In this study, the duration determined for administering liquid nitrogen to samples was divided into three times, namely 1 minute, 5 minutes, and 10 minutes, where there is still minimal data that can be obtained from the treatment of applying liquid nitrogen to bones or other structures using duration under 20 minutes. This collection of studies and protocols suggests that the minimum duration for the correct use of liquid nitrogen on chondrocyte cells is above 2 minutes. This can indirectly explain why, in this study, the treatment with a duration of 1 minute showed less significant results while the treatment with durations of 5 minutes and 10 minutes showed a substantial reduction in the number of chondrocytes. It is correct to conclude that the duration of liquid nitrogen exposure can significantly impact chondrocyte viability. Prolonged exposure can cause greater cell damage and death compared to shorter exposure. This suggests that it is important for medical professionals and researchers to take appropriate precautions and minimize the duration of liquid nitrogen exposure to maintain chondrocyte viability and cartilage tissue integrity.[17-19]

The relationship between the duration of liquid nitrogen administration - chondrocytes is known to have a correlation value of -0.858 with a p-value of 0.000; a correlation value of 0.858 indicates that the duration of liquid nitrogen administration and chondrocytes has a relationship with a very high category, the direction of the negative relationship suggests that the longer the liquid nitrogen administration will be followed by a decrease in chondrocytes. Meanwhile, with a value of p = 0.000 < 0.05 (α = 5%), it can be concluded that there is a significant relationship between the duration of liquid nitrogen administration and chondrocytes.

Research supports that long exposure to liquid nitrogen influences the viability and death of chondrocyte cells.[20] The duration of exposure to liquid nitrogen significantly reduced the number of chondrocytes in the 5-minute and 10-minute treatments. This research's weakness was that the reading accuracy level was lower because anatomical pathology specialists did not conduct the research results on cell count readings. Further research may help determine the optimal exposure duration for certain applications and develop better preservation techniques.

CONCLUSION

The liquid nitrogen administration significantly affects the number of chondrocytes in the cartilage of the Rattus norvegicus strain. The duration of the administration has a negative correlation with chondrocyte levels, which indicates that a decrease will follow a longer administration in chondrocytes.

Declaration by Authors

Ethical Approval: Approved by Health Research Ethics Committee Faculty of Medicine Universitas Brawijaya with number 20/EC/KEPK-PPDS/02/2023

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REFERENCES

1. Van Praag Veroniek VM, Rueten-Budde AJ, Ho V, et al. Incidence, outcomes and prognostic factors during 25 years of treatment of chondrosarcomas. Surg Oncol. 2018;27(3):402-408. doi:10.1016/j.suronc.2018.05.009
2. Igarashi K, Yamamoto N, Shirai T, et al. The long-term outcome following the use of frozen

autograft treated with liquid nitrogen in the management of bone and soft-tissue sarcomas. Bone Joint J. 2014;96-B(4):555-561. doi:10.1302/0301-620X.96B4.32629
3. Takata M, Sugimoto N, Yamamoto N, et al. Activity of bone morphogenetic protein-7 after treatment at various temperatures: freezing vs. pasteurization vs. allograft. Cryobiology. 2011;63(3):235-239. doi:10.1016/j.cryobiol.2011.09.001
4. J. Bickels, I. Meller, M. Malawer, The biology and role of cryosurgery in the treatment of bone tumors, Musculoskeletal cancer surgery, Kluwer Academic Publishers, 2004, pp. 135-146
5. Bickels J, Meller I, Shmookler BM, Malawer MM. The role and biology of cryosurgery in the treatment of bone tumors. A review. Acta Orthop Scand. 1999;70(3):308-315. doi:10.3109/17453679908997814
6. Charan J, Kantharia ND. How to calculate sample size in animal studies?. J Pharmacol Pharmacother. 2013;4(4):303-306. doi:10.4103/0976-500X.119726
7. Henry JP, Bordoni B. Histology, Osteoblasts. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557792/
8. Ghasemi A, Zahediasl S. Normality tests for statistical analysis: a guide for non-statisticians. Int J Endocrinol Metab. 2012;10(2):486-489. doi:10.5812/ijem.3505
9. Zhou Y, Zhu Y, Wong WK. Statistical tests for homogeneity of variance for clinical trials and recommendations. Contemp Clin Trials Commun. 2023;33:101119. Published 2023 Mar 31. doi:10.1016/j.conctc.2023.101119
10. Mishra P, Singh U, Pandey CM, Mishra P, Pandey G. Application of student's t-test, analysis of variance, and covariance. Ann Card Anaesth. 2019;22(4):407-411. doi:10.4103/aca.ACA_94_19
11. Mukaka MM. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. Malawi Med J. 2012;24(3):69-71.
12. Xia Y, Darling EM, Herzog W. Functional properties of chondrocytes and articular cartilage using optical imaging to scanning probe microscopy. J Orthop Res. 2018;36(2):620-631. doi:10.1002/jor.23757
13. Jaiswal AN, Vagga A. Cryopreservation: A Review Article. Cureus. 2022;14(11):e31564. Published 2022 Nov 16. doi:10.7759/cureus.31564
14. Chang T, Zhao G. Ice Inhibition for Cryopreservation: Materials, Strategies, and Challenges. Adv Sci (Weinh). 2021;8(6):2002425. Published 2021 Feb 1. doi:10.1002/advs.202002425
15. Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. Sports Health. 2009;1(6):461-468. doi:10.1177/1941738109350438
16. Alizadeh Sardroud H, Wanlin T, Chen X, Eames BF. Cartilage Tissue Engineering Approaches Need to Assess Fibrocartilage When Hydrogel Constructs Are Mechanically Loaded. Front Bioeng Biotechnol. 2022;9:787538. Published 2022 Jan 12. doi:10.3389/fbioe.2021.787538
17. Hooiveld M, Roosendaal G, Wenting M, van den Berg M, Bijlsma J, Lafeber F. Short-term exposure of cartilage to blood results in chondrocyte apoptosis. Am J Pathol. 2003;162(3):943-951. doi:10.1016/S0002-9440(10)63889-8
18. Freitas-Ribeiro S, Reis RL, Pirraco RP. Long-term and short-term preservation strategies for tissue engineering and regenerative medicine products: state of the art and emerging trends. PNAS Nexus. 2022;1(4):pgac212. Published 2022 Sep 30. doi:10.1093/pnasnexus/pgac212
19. Crisol M, Wu K, Congdon B Jr, et al. Chondrocyte Viability of Particulated Porcine Articular Cartilage Is Maintained in Tissue Storage After Cryoprotectant Exposure, Vitrification, and Tissue Warming. Cartilage. 2024;15(2):139-146. doi:10.1177/19476035221118656
20. Xia Z, Murray D, Hulley PA, Triffitt JT, Price AJ. The viability and proliferation of human chondrocytes following cryopreservation. J Bone Joint Surg Br. 2008;90(9):1245-1248. doi:10.1302/0301-620X.90B9.20652

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