THE NEW ARMENIAN MEDICAL JOURNAL
Vol.7 (2013), No 1, p. 99-103

# SHIFTS IN THE CONTENT OF SYNOVIAL FLUID TOTAL PROTEIN AS POSSIBLE CRITERIA FOR ESTIMATION OF THE POSTMORTEM INTERVAL (experimental study)

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*Received 9/21/2011; accepted in final form 12/12/2012*

***ABSTRACT***

*Until nowadays, the synovial fluid never became a study object for determining the postmortem interval. Meanwhile, it is not excluded that physical-and-chemical changes occurring in the synovial fluid might reflect specific stages of the regional autolytic process development in the main composite components of the joints synovial environment.*

*In the present study an attempt was made to establish dependence between the prescription of death coming and shifts in total protein contents determined by biuret method and the refractometry indices of knee joints synovial fluid in cows.*

*According to data obtained, in the synovial fluid of cow knee joints during the first 5 days after the slaughter an identical picture was observed regarding the changes in both total protein, and refractometry indices. In post-slaughter delayed period rather low indices of refractometry and total protein were recorded.*

*The performed research allows to draw a conclusion that total protein and refractometry indices of the synovial fluid, to a certain extent, might be considered as criteria for postmortem interval estimate. It is rather characteristic that in each study group studied indices might serve as criteria for time of death determination precisely for the given time interval.*

*In forensic medical practice the refractometry should be considered a relatively informative rapid method for estimation of the postmortem period in case when the synovial fluid is the study object.*

***Keywords:*** *prescription of death coming, synovial fluid, total protein, viscosity.*

## INTRODUCTION

The synovial fluid is a known composite component of the joints synovial environment considered as the terminal layer between the synovial membrane, articular cartilage, and subchondral bone [*Strukov A., Beglaryan A., 1963; Kopyeva T., 1980; Pavlova V., 1980*]. The synovial fluid serves as an indicator of the joint soft and hard tissues vital activity and is replenished by substances from the blood plasma, and, especially, by joint covering layer membrane secretion.

The synovial fluid has a very delicate and "adequate" response to the pathological process in the soft and hard tissues of the joints in variety of diseases of the musculoskeletal system [*Sinyachenko O., Ignatenko G., 2008*]. Many authors consider physical-and-chemical, cytological and enzymatic changes in the synovial fluid as additional criteria for laboratory diagnosis of connective tissue systemic diseases with immune disorders [*Strukov A., Beglaryan A., 1963; Nasonova V., 1976; Baggiolini M., 1979; Strukov A., Makarova O., 1982*], acute monoarthritis [*Panush R., 2002; Maury E., Flores R., 2006; Roll P. et al., 2007*], Salmonella [*Chou C. et al., 2004*], Borrelia [*Holl-Wieden A. et al., 2007*], Micoplasma [*Cole B. et al., 1971; 1975; Zilfyan A. et al., 1981; 1982; Johnson S. et al., 2007*] arthritis.

At the same time, until now the synovial fluid never became study object for defining the postmortem interval. Meanwhile, it is not ruled out that physical and chemical changes in the synovial fluid might reflect the particular stages of regional

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autolytic process development in composite components of the joints synovial environment.

It should be noted that nowadays for the synovial fluid studies upon musculoskeletal system lesions a variety of modern research methods is used, including such methods as polarization microscopy, cell culture, electrophoresis, radioimmunoassay and enzyme immunoassay, polymerase chain reaction, liquid chromatography, spectrophotometry, as well as immunohistochemical and cytochemical methods, etc. [S*inyachenko O., Ignatenko G., 2008*]. However, in forensic medicine the routine informative methods are more demanded for determining the time of death coming; these procedures do not require special skills, expensive equipment and reagents.

In this study we made an effort to establish the relationship between the postmortem time intervals and shifts in the content of total protein, including the refractometry indices estimated in the synovial fluid of cow knee joints. This approach was dictated by the following circumstance: prior to research on autopsy material, at which it might be impossible to determine the dynamics of physical and chemical changes in the synovial fluid, we considered it necessary to carry out such studies in animals.

## MATERIAL AND METHODS

The synovial fluid of the knee joints of 16 mature cows at the age of 5 years was the object of study. The animals were divided into four groups. Using the puncture needle, in the first group the synovial fluid of knee joints was removed immediately after slaughter, in the second group it was done on the day after slaughter, in the third group the fluid was drawn on the fourth day after the slaughter, and in the fourth group puncturing was performed on the fifth day after slaughter. As a result, the synovial fluid was extracted from four knee joints of every animal during different time intervals. Samples of animal knee synovial fluid of the first study group knee joints, i.e. specimens obtained immediately after the slaughter, were delivered in a cold container to the Scientific-Research Center of the Yerevan State Medical University after M. Heratsi. Samples of synovial fluid from animals of the second, third and fourth groups were extracted from resected knee joints and stored at room temperature for 2, 4, and 5 days, respectively.

All the samples were subjected to biochemical and refractometric analyses. The level of total protein was measured at a wavelength of 540 *nm* by biuret method and expressed in *g/l*.

During refractometry, a drop of settled (transparent) synovial fluid was applied on the refractometer definition prism with pasteur pipet. This operation was carried out fairly quickly, until the air bubbles might appear in the drop. Using the device screw we achieved a position when the view field of illuminated and dark parts boundary line passed through the point of intersection of crosswise arranged lines. After that, we took into account the values of refractometer scale (synovial fluid refractive index).

Studies were conducted on refractometer "RL-2" (Poland) and the portable refractometer "Inonas" ("Shibuya Optical", Japan). The results received from both refractometers were identical.

Statistical analysis was performed with Student's criteria with the help of IBM SPSS Statistic 20 program.

## RESULTS

The results of statistical analysis for total protein and refractometry indices of the synovial fluid obtained from cow knee joints are presented in the Table.

As obvious from the Table, in the synovial fluid

***TABLE.***

Indices of refractometry and total protein in the synovial fluid of cow knee joints at various time intervals after slaughter (n = 16)

| Study groups | Total protein | Viscosity |
|---|---|---|
| Group I | 43.5 ± 3.67 | 1.33983±0.000358 |
| Group II | 23.1 ± 2.91<br>$P_1$<0.0005 | 1.33622±0.000273<br>$P_1$<0.0005 |
| Group III | 33.9 ± 2.69<br>0.05>$P_1$>0.025<br>0.01> $P_2$>0.005 | 1.33773±0.00038<br>0.005> $P_1$<0.0005<br>0.005 $P_2$>0.0005 |
| Group IV | 24.6 ± 2.87<br>$P_1$<0.0005<br>0.025>$P_3$>0.01<br>0.4 >$P_4$>0.25 | 1.33631±0.000345<br>$P_1$<0.0005<br>0.005>$P_3$<0.0005<br>$P_4$>0.4 |

***NOTE:*** *$p_1$ – indices of the II, III and IV groups in relation to the first group;*
*$p_2$ – indices of the III group in relation to the II group;*
*$p_3$ – indices of the IV group in relation to the III group;*
*$p_4$ – indices of the IV group in relation to the II group..*

of cow knee joints during the first five days after slaughter, there was an identical picture in the content of total protein and indices of refractometry. Starting from the second day after slaughter and during the next three days the measured values of total protein and refractometry were much lower in comparison with those determined in the synovial fluid obtained from knee joints in the first study group animals that was conventionally considered as Control. On the second day after the slaughter indices of refractometry in the synovial fluid decreased 1.6 times, on the fourth – 1.3 times, and on the fifth day – 1.5 times.

A similar picture was observed upon analysis of total protein levels in synovial fluid of studied animals knee joints. The indices of total protein in the second study group were 1.9 times lower than those in the synovial fluid of knee joints of the first study group, while in the third group the indices were 1.3 times lower, and in the fourth one – 1.8 times lower than the same indices in the control group.

It should be noted that in animals of the third study group we identified the relatively high levels of total protein and refractometry indices compared to those observed in the synovial fluid of the second study group animals knee joints. At the same time, both studied parameters of synovial fluid in the animals of the fourth study group were lower than those determined in the synovial fluid of the third study group animals; the same indices did not significantly differ from those registered by us in the synovial fluid of the second study group animals knee joints.

Research results showed that in the synovial fluid of knee joints during five days after slaughter the similar dynamics shifts were observed both in the content of total protein and refractometry indices, as illustrated in the Figure.

Moreover, two-factor statistical analysis of data obtained in our studies for all time intervals revealed the direct strong correlation dependence between studied parameters.

## DISCUSSION

Based on the research thus performed, a conclusion might be drawn that indices of the synovial fluid total protein and refractometry, to a certain extent, should be considered as criteria for determining the prescription of death coming, because starting from the second day after the slaughter of animals there was observed a dynamics of shifts in distribution of the studied parameters, which was characterized by their decrease in synovial fluid in all subsequent periods of study. It is significant that the tested parameters in each study group might be considered as criteria for prescription of death coming for the particular period. These are indices of total protein and refractometry determined in the synovial fluid of animal knee joints immediately after slaughter and on the fourth day after slaughter. However, both indices were less informative for comparison of our study results on the synovial fluid extracted from knee joints of animals slaughtered on the second and fifth days of the experiment.

As shown above, after the slaughter in the synovial fluid of knee joints direct correlation was found between the indices of refractometry and total protein in all periods of study. In the available literature we failed to find similar information for determination of both indices characterizing the total protein levels in the synovial fluid. At the

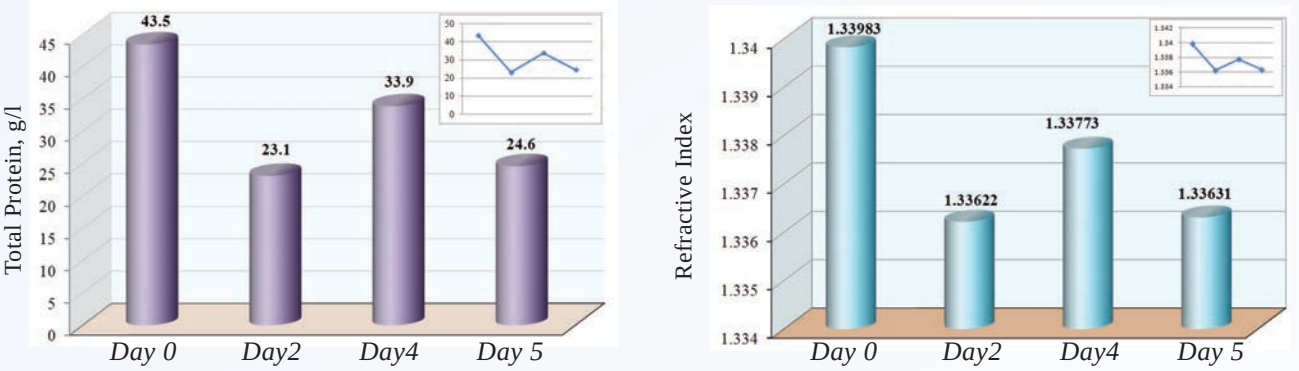


***Figure.*** *Dynamics of shifts in total protein and refractometry indices in synovial fluid of animal knee joints at various time intervals after slaughter.*

same time, a similar pattern was found in the blood serum. In particular, the manuals for refractometers "Reichert TS 400" (Germany) and "RF-22" (Russia) contain very informative tables clearly presenting correlation dependence between the refractive indices (up to a thousandth part) and protein indices for the corresponding refractive indices (up to ten thousandth part).

In this context, in our opinion, it is of interest to mention the investigation on blood plasma of 56 dogs and 84 cats performed in order to compare the results of refractometry and total protein levels [*Briend-Marchal A. et al., 2005*]. The results were comparable and the authors believed that refractometry might be a good substitution equivalent method in the study on protein concentration in blood plasma of mammals.

Thus, the pattern observed in control healthy group of animals, in our opinion, allows us to introduce refractometry in specialized forensic laboratories as a relatively informative method for determination of total protein precisely in the synovial fluid. At the same time, we can rule out the possibility of approbation and usage of more cumbersome and relatively expensive methods of total protein content determination in the synovial fluid during laboratory daily activities (biuret test, Lowry method, precipitation of proteins by sulfosalicylic acid), especially when forensic doctors are requested to determine the exact time of death.

In forensic medicine the refractometry should be regarded as a relatively informative rapid method for determining the time of death, when synovial fluid should serve the study object. Currently, there are relatively inexpensive, but high quality portable refractometers, which do not require special reagents, long time and skills for performance. To a certain extent, the usage of synovial fluid refractometry in forensic practice should be based on the results of pathologic (postmortem) and forensic diagnosis. First of all, it refers to diseases of musculoskeletal system, in which, as stated above, with the development of regional pathological changes in composite components of the joints synovial environment, the most significant activation of catabolic reactions occurs in the synovial fluid; this latter entails changes in physical and chemical properties. Therefore, in similar diseases, the synovial fluid cannot be the object of study for estimation of postmortem time intervals.

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