COMPARATIVE EVALUATION OF HISTOLOGICAL ANALYSES OF EXPERIMENTAL STEATOSIS, PERFORMED BY TRAINED ACADEMICS AND PATHOLOGIST

https://doi.org/10.56238/sevened2025.021-002

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ABSTRACT

The participation of academics in the research process is limited by the lack of specific training, which reduces its effectiveness and engagement. This study evaluated the performance of medical students trained in the histological analysis of nonalcoholic fatty liver disease (NAFLD) in an animal model, comparing their analyses with those of a pathologist. The research was carried out with seven medical students from the State University of Ponta Grossa (UEPG), who analyzed liver tissue samples from rabbits, using the previously validated histological scoring system. The results showed that the steatosis and balloonization analyses performed by the students were similar to those of the pathologist, with no statistically significant difference ($p>0.05$). However, the analysis of lobular inflammation showed a significant difference ($p<0.001$), suggesting the need for

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more training time for this specific alteration. It is concluded that, with adequate training, medical students can perform histological analyses of NAFLD in animal models, enhancing institutional scientific production.

Keywords: Nonalcoholic Fatty Liver Disease. DHGNA. Academic training. Hepatic steatosis. Medical education. Animal model.

INTRODUCTION

The participation of academics in the research process as a whole is limited mainly by the lack of specific training, which is a barrier often seen by them as insurmountable. Without adequate training, the participation of the student becomes something close to the provision of services that is merely mechanical and peripheral, discouraging and ineffective. From the orientation and training of medical students who have already completed the basic curricular disciplines, (from the 4th year onwards), in the context of the proposed research, it is possible not only to insert them objectively and productively in research of a certain line, but also to stimulate them to develop aptitudes. On the other hand, the institution's research activities involving trained academics will have technical refinement and consequently culminate in more expressive scientific production in number and quality.

The scoring system for the histological analysis of nonalcoholic fatty liver disease (NAFLD) validated by a previously published article (Kleiner, 2005) has been used in randomized clinical trials (Sanyal, 2010; Neuschwander-Tetri, 2015; Lavine, 2011) and experiments (Sturzeneker, 2011; Sturzeneker, 2019). This scoring system allows the objective completion of histological analyses by numerical expression, facilitating the conclusion as well as their comparison. Steatosis, lobular inflammation, hepatocyte ballooning degeneration (balloonization), and fibrosis are the basic histopathological changes that can be found in NAFLD in humans (Chalasani, 2018). Nonalcoholic steatohepatitis (NASH) is defined by the concomitant presence of the first 3 histopathological changes cited, with or without associated fibrosis (Chalasani, 2018) and its presence can be estimated by the unweighted sum of the steatosis, lobular inflammation, and balloonization scores (Kleiner, 2005).

To date, there are no adequately publicized studies evaluating the performance of academics trained in histological analysis of liver tissue samples from an animal model of NAFLD. However, effectively enabling the participation of academics in this process can culminate in the expansion, with quality, of the production of experiments by teaching and research institutions and consequently expand the specific knowledge of both academics and research professors. In this rational line, the study in question was carried out with the objective of evaluating the performance of medical students trained in histological analysis of liver tissue samples from an animal model of NAFLD, comparing the analyses of the respective students with the standard analysis of the pathologist.

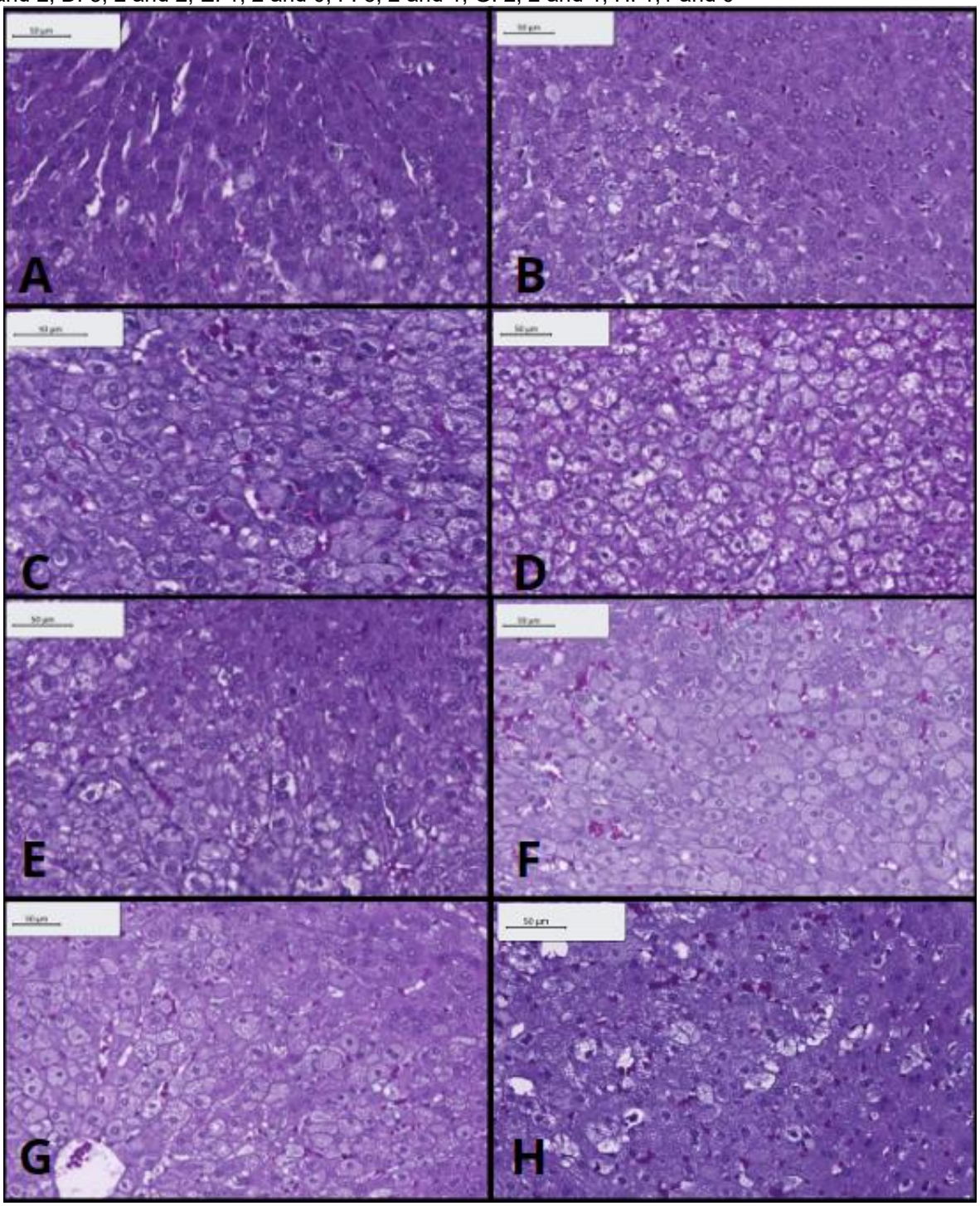
OBJECTIVE

To compare the histological analyses of experimental steatosis, performed by trained medical students of our institution, based on the scoring system for histological analysis of NAFLD, with the same analysis performed by a specialist in the field (pathologist). To contribute to the validation of the histological analysis of experimental NAFLD, carried out by trained academics and consequently to stimulate the creation of a training program for academics in this context.

METHOD

This study was previously conceived and registered as a teaching research at the State University of Ponta Grossa (UEPG), carried out between February and July 2024. The processing of tissue samples was carried out by the experimental pathology laboratory of the Pontifical Catholic University of Paraná (PUC-PR). The specialized analysis, selection of the material and assembly of the material for classes and analyses were carried out by the pathologist and professor involved in the study and the students involved are currently undergraduate students of the medical course at UEPG: 3 students from the 6th and 4 students from the 4th year. Slides stained with hematoxylin and eosin were made from liver tissue samples of albino male rabbits (Oryctolagus cuniculus), of *the New Zealand lineage*, from the Central Vivarium of PUC-PR, used in an experiment previously carried out at the respective institution, approved by the ethics committee for the use of animals under opinion number 240/08 (Sturzeneker, 2011) and recently submitted to expanded reanalysis (Justus, 2024). After selecting slides with liver tissue samples representative of 3 histological alterations (steatosis, lobular inflammation and balloonization), as well as the absence of the respective basic alterations, photomicrographs were performed and made available using the Zeiss Zen Lite computer program. The students were guided by the pathologist, and the analyses were performed based on the scoring system for histological analysis of NAFLD (Table 1), except for ballooning, which was divided into 3 levels (scores 0 to 2) in order to facilitate the analysis of the students (Kleiner, 2005). Subsequently, the basic images were made available and the doubts related to the analysis were clarified subsequently by contact with the advising professors. Subsequently, 33 photomicrographs were made available containing the histological alterations in all their degrees (score 0 to 3), randomly distributed for the final analysis of the students, which was completed in 3 to 4 weeks after the first formal orientation (Figures 1 and 2).

Figure 1. Photomicrographs of slides stained with hematoxylin and eosin, magnification of 200 X, with different scores (0 to 3), respectively for steatosis, ballooning, and lobular inflammation. A: 0.1 and 0; B: 1, 1 and 1; C: 2, 2 and 2; D: 3, 2 and 2, E: 1, 2 and 0; F: 3, 2 and 1; G: 2, 2 and 1; H: 1,1 and 0



Source: composition of the authors.

The results of the histological analyses of each parameter (steatosis, lobular inflammation and balloonization) were compared using the Kruskal-Wallis test, as an alternative to one-factor analysis of variance (ANOVA). Multiple comparisons were performed using the Dwass-Steel-Critchlow-Fligner test. The data were analyzed using the Jamovi computer program version 2.3.28. Values of $p<0.05$ were considered significant.

Table 1 – Main parameters of the scoring system for histological analysis of NAFLD

Alteration	Definition	Score
Degree of steatosis	<5%	0
	5 to 33%	1
	>33 to 66%	2
	>66%	3
Lobular inflammation	Unfocused	0
	<2 Spotlights/Field(200 X)	1
	2-4 Focuses/Field(200X)	2
	>4 Spotlights/Field(200X)	3
Ballooning	Absent	0
	Few cells	1
	Many cells	2

Source: composition of the authors.

RESULTS

The comparison of the results of the histological analyses of each parameter of interest (steatosis, lobular inflammation and balloonization) performed by the pathologist and the 7 students involved showed no significant difference in relation to steatosis (p=0.765), and the same was observed in relation to balloonization (p=0.120). Comparison 2 to 2 showed similarity between all steatosis and balloonization analyses (Table 2). However, there was a significant difference between the analyses and lobular inflammation, with a p<0.001 value. The comparisons, 2 to 2, between the analyses of the pathologist and the students were similar, except for the one made with student 2, whose value was significant: p=0.003 (Table 2).

Table 2. Multiple comparisons 2 to 2

2 TO 2 COMPARISON		HISTOLOGICAL ALTERATION – P-VALUE		
		Steatosis	Ballooning	Lobular Inflammation
Pathologist	Acad.1	0.998	1.000	0.873
Pathologist	Acad.2	0.999	1.000	0.003
Pathologist	Acad.3	0.987	0.991	1.000
Pathologist	Acad.4	1.000	1.000	0.973
Pathologist	Acad.5	0.984	0.215	0.013
Pathologist	Acad.6	1.000	1.000	0.928
Pathologist	Acad.7	1.000	0.996	1.000

Source: composition of the authors.

Comparisons 2 to 2 between students had a significant value as shown below: between students 1 and 2 (p<0.001), 1 and 5 (p<0.001), 2 and 4 (p<0.001), 2 and 6 (p=0.038), 2 and 7 (p=0.004), 4 and 5 (p<0.001) and between students 5 and 7 (p=0.011) (Table 3).

Table 3. Multiple comparisons 2 to 2

2 TO 2 COMPARISON		HISTOLOGICAL ALTERATION – P-VALUE		
		Steatosis	Ballooning	Lobular Inflammation
Acad.1	Acad.2	0.918	1.000	<.001

Acad.1	Acad.3	0.779	0.993	0.862
Acad.1	Acad.4	0.991	1.000	1.000
Acad.1	Acad.5	0.799	0.194	<.001
Acad.1	Acad.6	1.000	1.000	0.168
Acad.1	Acad.7	0.994	0.997	0.884
Acad.2	Acad.3	1.000	0.983	0.005
Acad.2	Acad.4	1.000	1.000	<.001
Acad.2	Acad.5	1.000	0.165	0.716
Acad.2	Acad.6	0.996	1.000	0.038
Acad.2	Acad.7	0.999	0.991	0.004
Acad.3	Acad.4	0.997	0.998	0.970
Acad.3	Acad.5	1.000	0.746	0.019
Acad.3	Acad.6	0.971	0.998	0.930
Acad.3	Acad.7	0.987	1.000	1.000
Acad.4	Acad.5	0.996	0.313	<.001
Acad.4	Acad.6	1.000	1.000	0.315
Acad.4	Acad.7	1.000	1.000	0.980
Acad.5	Acad.6	0.966	0.326	0.109
Acad.5	Acad.7	0.990	0.518	0.011
Acad.6	Acad.7	1.000	0.999	0.876

Source: composition of the authors.

DISCUSSION

The histological analysis of NAFLD based on the scoring system (Kleiner, 2005) allows the standardization of the result and consequently reduces interobserver variability. Therefore, it enables the inclusion of adequately oriented academics in the process of histological analysis, which, in this context, expands the productive potential of the institution.

In our study, the 3 basic histological alterations that characterize NAFLD in humans (steatosis, lobular inflammation, and hepatocyte ballooning) were analyzed and compared, based on a previously validated analysis system (Kleiner, 2005), the animal model material used was previously published with other forms of approach in indexed journals (Sturzeneker, 2011; Justus, 2024). Therefore, in general terms, the study in question has value, from a practical point of view, for research-oriented institutions.

The steatosis analyses of the pathologist and the 7 students were similar ($p=0.765$), and the same was observed in relation to the balloonization analyses ($p=0.120$). The 2 to 2 comparison reinforced this similarity, characterizing a non-significant interobserver variability. This result leads us to infer that there was greater ease of assimilation of the learning process, outlined as a method, for these two histological alterations. However, there was a significant difference in relation to the analysis of lobular inflammation ($p<0.001$), demonstrated in the 2 to 2 comparison between the pathologist and student 2 ($p=0.003$) and between students mainly involving student 2 (Table 2).

Possibly, the training process of the students was inadequate only for the analysis of lobular inflammation, probably because it requires a more detailed characterization and,

therefore, with greater complexity than the others analyzed (steatosis and balloonization). Therefore, for this alteration, a longer period of training with greater exploration of the different inflammation scores is necessary and would probably determine the same outcome as the other alterations analyzed. It is necessary to emphasize that all the students who volunteered to participate in the study were included (7 students) and none of them were excluded for the finalization and analysis of the results, which could be different if the analysis of student 2 was excluded.

Regarding the fact that most of the training process was done remotely and the entire analysis process was carried out by means of photomicrographs, i.e., "digital slides", which can be called digital pathology, in a meta-analysis that selected 24 validation studies, totaling 19,468 comparisons between digital and physical slides, the overall and complete agreement rate observed was 98.3% and 92%, respectively (Azam, 2021). Regarding the teaching process, in human medicine, the replacement of conventional pathology by digital pathology has been reported (Bertram, 2017). The use of digital slides or the projection of the image of the slides on screens has been widely used in educational institutions, which breaks the limiting barrier imposed by simple analysis via optical microscopy (Al-Janabi, 2012). In a specific study, compared to traditional methods, digital pathology had significantly positive acceptance by academics, mainly due to its practicality, reduction of technical failures, and expansion of access (Alves, 2021).

CONCLUSION

Histological analysis of steatosis and hepatocyte ballooning degeneration (balloonization) of an animal model of NAFLD (hypercholerolemic rabbits) can be performed by adequately trained medical students. However, for the analysis of lobular inflammation, there is a need for additional training time properly adjusted for each student involved. Therefore, it can be inferred that with the appropriate training method, the basic histological alterations of human NAFLD, developed in an animal model, can be analyzed by adequately trained medical students, which can significantly expand the experimental production in this context.

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