Correlation between sonographic features and nuclear morphometric parameters in parotid gland tumors

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Authors: D. Obad Kovacevic, J. Popic, I. Kardum Skelin, G. Kai#, B. Jeli#
Puškaric; Zagreb/HR
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Aims and objectives

Salivary gland tumors are relatively rare, most of them are benign and found in parotid gland [1,2]. Ultrasound (US) is used for tumor detection, characterization and guiding fine-needle aspiration cytology (FNAC) [1,3,4]. US and FNAC together, permit differentiation of a benign from a malignant tumor in the majority of cases [1,4,5].

Digital cytomorphometry aids in the differentiation between malignant and benign tumors [6]. Malignant cells generally have bigger and more irregular shaped nuclei compared to benign cells [7-9]. Whole cells morphometric parameters were proved to be less valuable in distinguishing between benign and malignant cells [7,1].

US feature that suggests benignancy is posterior acoustic enhancement [11]. US features that are more suggestive for malignancy are heterogeneous echotexture, irregular margins and regional lymph node enlargement [1,2,11].
Methods and materials

Sonography

US examination of 64 parotid gland tumors was performed using a 7.5-MHz linear-array transducer. Four sonographic tumor parameters were recorded: echotexture, margins regularity, distal acoustic enhancement, and regional lymph node enlargement.

The echotexture of the tumor was evaluated as homogeneous or heterogeneous. The appearance of the tumor margins was evaluated as regular or irregular. The distal acoustic enhancement was recorded as present or absent. The regional lymph node enlargement (>10 mm in size, with distortion of the internal architecture) was evaluated as present or absent.

FNAC and digital cytomorphometry

All patients signed a written informed consent before FNAC. FNAC was performed with a 22-23 gauge (G) needle. The aspirates were stained with May-Grünwald Giemsa stain. FNAC results were: benign, indeterminate and malignant. Benign and malignant FNAC results were included in this study. Malignant lymphomas and tumors with indeterminate FNAC findings were not included in this study.

The SFORM System consisting of a microscope, a high-resolution colour TV camera and a PC-compatible computer was used for nuclear morphometry. The analysis was performed on 100 tumor cells, at magnification x1000. The outlines of the nucleus and cell membrane were traced and results were logged in tables. Nuclear morphometric parameters of 6400 nuclei were included in this study.

Eleven nuclear morphometric parameters were recorded: area, perimeter, convex area, maximal radius, minimal radius, length, breadth, form factor, (FF=4#area/r²), elongation factor (EF=breadth/length), area symmetry factor (ASF=larger/smaller part of area, after drawing the largest diameter of the nucleus), and perimeter symmetry factor (PSF=larger/smaller part of perimeter, after drawing the largest diameter of the nucleus). Areas and perimeters were expressed in µm² and µm, respectively. The form factors were calculated according to given formulas and were ≥ 1 ≤.

Sonographic features and quantitative nuclear morphometric parameters were compared.
Statistical analysis was performed using Statistica Ver. 7.1 statistical package (StatSoft, Inc., Tulsa, USA). The significance of differences between tumors was assessed by nonparametric Kruskal-Wallis test and #2 test. $P$ values of 0.05 or less were considered statistically significant.
Results

The study included 64 FNAC diagnosed parotid gland tumors: 42 (66%) benign tumors and 22 (34%) malignant tumors.

Among tumors with irregular margins FNAC revealed 11 malignant and 1 benign tumor (p<0.05). Among tumors with heterogeneous echotexture FNAC found 17 benign and 17 malignant tumors (p<0.05). 31 benign and 6 malignant tumors had posterior acoustic enhancement (p<0.05). 12 malignant and 2 benign tumors were associated with regional lymph node enlargement (p<0.05).

Sonograms are shown in Figures 1 and 2.

Tumors with irregular tumor margins had significantly higher values of the following nuclear morphometric factors: area, perimeter, convex area, minimal radius, maximal radius, length, and breadth compared to tumors with regular tumor margins (p<0.05). FF, EF, and PSF were significantly closer to the value of 1 in tumors with regular tumor margins (p<0.05).

Tumors associated with regional lymph node enlargement had significantly higher values of: area, perimeter, convex area, minimal radius, maximal radius, length and breadth, compared to tumors that are not associated with regional lymph node enlargement (p<0.05). FF, EF, ASF, and PSF were significantly closer to the value of 1 in tumors that are not associated with regional lymph node enlargement (p<0.05).

Tumors with heterogeneous echotexture had significantly higher values of: perimeter, convex area, minimal and maximal radius, compared to homogeneous tumors (p<0.05). FF, EF, and ASF were significantly closer to the value of 1 in homogeneous tumors (p<0.05).

Tumors with posterior acoustic enhancement had significantly lower values of the following nuclear morphometric factors: perimeter, area, convex area, and minimal radius, compared with tumors that do not show posterior acoustic enhancement (p<0.05). FF, ASF and PSF were significantly further from the value of 1 in tumors that do not show posterior acoustic enhancement (p<0.05).

Digital image nuclear morphometry is shown in Figures 3 and 4.

Results are summarized in Tables 1 and 2.
Fig. 1: Sonogram of the parotid gland shows hypoechoic, slightly inhomogeneous tumor with regular tumor margins and strongly expressed posterior acoustic enhancement (arrows). FNAC diagnosis was Warthin`s tumor.

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Fig. 2: Sonogram of the parotid gland shows a hypoechoic, heterogeneous mass with irregular tumor margins (arrows). FNAC diagnosis was anaplastic carcinoma.

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Fig. 3: Digital morphometric analysis of cytologic aspirate of cystadenolymphoma: measurement of area symmetry factor and perimeter symmetry factor (May-Grünwald Giemsa, x1000).

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Fig. 4: Digital morphometric analysis of cytologic aspirate of squamous cell carcinoma: measurement of area symmetry factor and perimeter symmetry factor (May-Grünwald Giemsa, x1000).

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### Table 1: Correlation between nuclear morphometric parameters and sonographic features of parotid gland tumors (echotexture and posterior acoustic enhancement)

n, number of tumors; X, mean value; SD, standard deviation; AREA, nuclear area; PERIMETER, nuclear perimeter; MINR, nuclear minimal radius; MAXR, nuclear maximal radius; CONVA, nuclear convex area; LENGTH, nuclear length; BREADTH, nuclear breadth; FF, nuclear form factor; EF, nuclear elongation factor; ASF, nuclear area symmetry factor; PSF, nuclear perimeter symmetry factor; P, the level of significance; NS, not significant.

*Values of the areas were expressed in µm², values of perimeters, radius, length and breadth were expressed in µm. Form factors were calculated according to the given formulas, and were ≥ 1 ≤.
Table 2: Correlation between nuclear morphometric parameters and sonographic features of parotid gland tumors (regional lymph node status and the regularity of tumor margins)

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Conclusion

The main tasks of US of parotid gland tumors are: detect tumor, differentiate between benign and malignant tumors, and guide FNAC [1,4,5].

FNAC task is to distinguish between benign and malignant tumors [3]. Digital cytomorphometry increases the accuracy of FNAC by turning qualitative data into quantitative values, using the occurrence of quantitative abnormalities of nuclear phenotype in tumors [6-10].

Although there are studies that deny the role of US in the differentiation between benign and malignant tumors, [12] the majority of studies agree that US can distinguish between benign and malignant tumors [1,2,1]. FNAC is a powerful diagnostic tool in the field of salivary gland tumors and the diagnostic accuracy of US-guided FNAC is similar to that of core needle biopsy [5].

In the literature, there are only a few studies on digital cytomorphometric analysis of parotid gland tumors [6,7]. On the other hand, there are many articles dealing with digital cytomorphometry of tumors of other organs [8-10].

Because malignant nuclei are generally bigger, more elongated or irregular shaped, previous cytomorphometric studies found higher values of some nuclear morphometric parameters in malignant tumors, compared to benign tumors, [6-10] which was confirmed in this study. In the previous morphometric studies, [6-10] the values of measured nuclear form factors in malignant cells were significantly further form the value of 1, compared with benign cells, which was also confirmed in this study.

Taking into account that posterior acoustic enhancement is more common in benign tumors and that heterogeneous echotexture, irregular margins, and regional lymph node enlargement are more common in malignant tumors, results obtained in this study are compatible with the results of previous studies [1,2,4,11]. Nuclear perimeter, convex area, minimal radius, and FF were the most predictive for malignancy, which agrees with some previous studies [6-10].

The existence of significant correlations between nuclear morphometric parameters and sonographic parameters confirms that the sonographic appearance of parotid gland tumors reflects the tumor appearance at the cellular/nuclear level.
Personal information

Dragica Obad Kovacevic PhD, Department of Diagnostic and Intervention Radiology, University Hospital Merkur, Zagreb, Croatia; dragica.obad.kovacevic@gmail.com

Jelena Popic Ramac PhD, Department of Diagnostic and Intervention Radiology, University Hospital Merkur, Zagreb, Croatia; jelena.popic.ramac@gmail.com

Ika Kardum-Sklelin PhD, Department of Clinical Cytology and Cytogenetics, University Hospital Merkur, Zagreb, Croatia;
ikardum@hi.t-com.hr

Biljana Jelic-Puskaric MD, Department of Clinical Cytology and Cytogenetics, University Hospital Merkur, Zagreb, Croatia;
biljana.jelic.puskaric@zg.t-com.hr

Gordana Kaić PhD, Department of Clinical Cytology and Cytogenetics, University Hospital Merkur, Zagreb, Croatia;
gordana.kaic@zg.t-com.hr
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