Evaluation of Features of Adrenal Adenomas and Nonadenomas Using Dynamic Contrast-Enhanced CT Biomarkers

Xifu Wang, MD a, 1, Xizhong Dong, BS b, 1, Tingting Huang, MS c, Jie Meng, BS d, Yuanxun Kuang, MS c, Jiwen Kang, BS e, Renju Bai, MD d, *, Zhaojun Li, MD a, *

a Department of Radiology, Shanghai General Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China; b Department of Radiology, Weifang People’s Hospital, Shandong, China; c Department of Radiology, Jiading Jiangqiao Hospital, Shanghai, China; d Department of Radiology, Tianjin Medical University General Hospital, Tianjin, China

Received July 15, 2021; revision received November 10, 2021; accepted November 12, 2020

Objective: To investigate the correlation between the DCE-CT imaging biomarkers and histological biomarkers of tumor angiogenesis in adrenal adenomas and non-adenomas for the enhancement mechanism of DCE-CT.

Methods: Forty-two patients with 45 adrenal masses including 27 adenomas and 18 non-adenomas diagnosed pathologically were enrolled in this study. The features of DCE-CT (imaging biomarkers) and tumor angiogenesis (histological biomarkers) in adrenal masses were evaluated, and their correlations were explored.

Results: The enhanced features of DCE-CT in adrenal masses were classified: rapid washout group and slow washout group. Type A and C of time density (TD) curves, relative washout rate (Washr) ≥34%, and absolute washout rate (Washa) ≥43% belonged to the rapid group. In contrast, type B, D and E, Washr <34%, and Washa <43% belonged to the slow group. There was significant difference between the biomarkers of DCE-CT in adrenal masses. The rapid group was mainly found in adenomas, whereas the slow was mainly present in nonadenomas. The tumor angiogenesis, histological biomarkers, including microvessel density (MVD), vascular endothelial growth factor (VEGF), and microvascular ultrastructures demonstrated significant difference between the rapid and the slow washout group revealed by DCE-CT. The MVD and VEGF expression in rapid group were remarkably higher than those in slow group. Meanwhile, the tumor angiogenesis was also significantly different between adenomas and nonadenomas. The MVD and VEGF expression were also significantly higher in adenomas than those in nonadenomas. Furthermore, different microvascular ultrastructures were identified between adenomas and nonadenomas, which were in accordance with those between the rapid and the slow group. Microvascular ultrastructures in adrenal adenomas and/or the rapid group showed regular lumens and nonstenosis; more pinocytotic vesicles and fenestrations of endothelium; widening of the intercellular space; uniform thinning and better integrity of basal membrane; regular and uniform thinning, along with less stroma of extra vessel space. In comparison, opposite microvascular ultrastructures, in adrenal nonadenomas and/or the slow group.

Conclusion: The close correlation of DCE-CT imaging biomarkers and histological biomarkers of tumor angiogenesis was found between adrenal adenomas and nonadenomas. Tumor angiogenesis in adrenal adenomas and nonadenomas were shown the different enhancement characteristics at DCE-CT.

Key words: Computer Tomography; Dynamic Contrast-Enhancement; Biomarker; Tumor Angiogenesis; Adrenal Adenomas

Advanced Ultrasound in Diagnosis and Therapy 2021; 04: 304-312 DOI: 10.37015/AUDT.2021.210020

1 Xifu Wang and Xizhong Dong contributed equally to this work.

* Corresponding author: Department of Radiology, Shanghai General Hospital, Shanghai Jiaotong University School of Medicine, 100 Haining Road, Hongkou District, Shanghai, China
e-mail: lzj_1975@sina.com (ZJ Li) and cjr.bairenju@vip.163.com (RJ Bai).

2576-2508 ©AUDT 2021 • http://www.AUDT.org
This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license, which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.
Adrenal masses are often detected incidentally on routine abdominal computed tomography (CT) examinations. Up to 5% of abdominal CT scans performed for reasons other than suspected adrenal disease may demonstrate an adrenal mass [1,2]. In patients with incidental adrenal masses, clinical decision-making on whether to perform biopsy, surgery or follow-up raises problems, particularly in patients with a history of extra-adrenal malignancy because it is usually impossible to differentiate an adrenal adenoma from a nonadenoma [1]. In particular, hyperattenuating adrenal masses, on the basis of standard morphological features, are particularly difficult to identify [2].

Fortunately, previous studies have reported the washout characteristics of adrenal lesions using dynamic contrast enhanced (DCE) computed tomography (CT) [1,3-11]. Washout refers to the decrease in the attenuation values in the adrenal mass at variable delay time points following the intravenous bolus injection of contrast material for DCE-CT. An adrenal adenoma exhibits a greater washout of contrast material than nonadenomas. Thus, the washout rate of DCE-CT is a useful method for differentiating adrenal adenomas from nonadenomas, which is accepted as the choice of diagnosis for adrenal adenoma.

However, to the best of our knowledge, reports correlating enhancement mechanisms with DCE-CT diagnostic performance are rare. Szolar et al. only speculated that potential histopathological explanations of the different angioarchitectures of adenomas and nonadenomas might be related to the preservation of cellular membrane integrity and the smaller expansion of effective extracellular space due to tumor infiltration in adrenal adenomas [11]. Thus, the purpose of our present study was to explore the correlation between the enhanced features of the DCE-CT imaging biomarkers and histological biomarkers of tumor angiogenesis in adrenal adenomas and nonadenomas and to illuminate this enhancement mechanism.

Materials and Methods

Patient population

This study was approved by the institutional review board and was compliant with Health Insurance Portability and Accountability Act guidelines. All participants provided written informed consent.

Forty-two patients with 45 adrenal masses were consecutively examined using DCE-CT included in this study. These patients consisted of 16 male and 26 female patients (mean age, 43.7 ± 11.6 years; age range, 9-64 years). A final diagnosis of individual masses was confirmed by surgery and pathology. These 45 masses comprised of 27 adenomas and 18 nonadenomas, which involved 23 left and 22 right adrenal glands. Of the 42 patients, two patients had four adrenal adenomas (two per patient), and one patient had bilateral nonadenomas. The mean axial diameter of the masses was 3.5 ± 2.1 cm (range, 1-9.9 cm). The nonadenomas were identified as follows: pheochromocytoma (n = 11), metastases (n = 3), cortical carcinoma (n = 1) and neurogenic tumors (n = 3).

CT protocol

The CT scans were performed using commercial Hispeed CT/i scanning equipment (GE Medical Systems, Milwaukee, WI, USA). The imaging parameters for dynamic contrast-enhanced CT examinations included 3.0-5.0-mm reconstruction slice thickness, 1:1 pitch, 1-sec rotation time, 120 kVp and 220 mA. Each scan (acquisition time, 18-24 seconds) was obtained with the patient in full inspiration to optimize the reproducibility of the starting measurements. The DCE-CT images were obtained before and after an intravenous administration of 100 ml-120 ml of Ultravist 370 (Bayer Schering Pharma, Berlin, Germany) or Omnipaque 350 (Nycomed/GE Healthcare, Milwaukee, WI, USA) at a rate of 2.5 ml/sec using a power injector. The six-phase DCE-CT scan delay time points included 0.5-, 1-, 2-, 3-, 5-, and 7-min, in reference to the beginning of contrast material administration.

Image and data analysis

The time density (TD) curves were obtained based on the absolute CT attenuation value at each delay time point. The TD curves of masses were classified into the following five types. Type A represents a rapidly rising slope (wash-in) during the early phase (≤1 min), followed by a rapid washout phase after the peak enhancement is achieved. Type B represents a rapidly rising slope during the early phase (≤1 min) that is followed by smooth decay or a plateau in the latter portion. Type C represents a slowly rising slope during the early phase (≥2 min), followed by rapid washout phases after the peak enhancement. Type D represents a slowly rising slope during the early phase (≥2 min), followed by smooth decay or a plateau in the latter portion. Finally, Type E represents a slow and gradual increase during the scanning period.

A circular or ovoid region-of-interest (ROI) cursor was used to measure the CT attenuation values. The ROI cursor covered at least one-half of the area of the adrenal mass at the corresponding level of each CT image, excluding cystic, necrotic, calcified and hemorrhagic regions. The peripheral areas of the adrenal lesion were avoided to reduce partial volume averaging.
The CT attenuation value of each lesion was measured at least twice by two experienced radiologists who were unaware of the clinical and pathological information for each patient and who worked independently (without consultation). If two radiologists did not coincide in their determination of the measurement, another radiologist made the final decision. The measurements obtained by the two radiologists were averaged, and the mean CT attenuation number was recorded. The relative and absolute washout rate were calculated separately, using the following equations: a) Washr = \[\frac{D_{\text{max}}-D_{\text{post}}}{D_{\text{max}} \times 100\%}\] and b) Washa = \[\frac{(D_{\text{max}}-D_{\text{pre}})-(D_{\text{post}}-D_{\text{pre}})}{(D_{\text{max}}-D_{\text{pre}}) \times 100\%}\] [1, 5, 8, 9, 11]. Dmax is the enhancement peak of the CT attenuation value in adrenal masses following the administration of contrast material; Dpre is the CT attenuation value at different time delays after the enhancement peak of the mass; Dpost is the unenhanced CT attenuation value of the masses.

**Microvascular density (MVD) and vascular endothelial growth factor (VEGF)**

Surgically resected specimens were obtained, and tumor samples were prepared for pathological examination from the same approximate location and in the same orientation as the analyzed DCE-CT scans. Tumor specimens were fixed in 10 % formalin and then embedded in paraffin. These specimens were cut into 4-mm sections, with the intention of obtaining the closest match between the ROIs and the examined specimens.

Three consecutive sections were collected from each tumor for immunohistochemistry analyses, including MVD and VEGF. Two-step immunohistochemistry was performed using the immunoglobulin-peroxidase method. The immunochemical images were acquired using the CMIAS multifunctional image analysis system and AGFA-II scanner (Image Center of Beijing University of Aeronautics & Astronautics, Beijing, China). The cells staining positive for the endothelial cell marker CD31 had brown or yellow-brown granules, microvessels with positive-staining cells without background were marked, and the MVD was quantified. The criteria for vessel counting were established by Weidner et al., and microvessels with identifiable positive-staining cells or cell clusters served as blood vessels [12]. Sections were observed at low magnification (× 40), and three hot areas with highest blood vessel density were recorded at a higher magnification (× 200; 0.739 mm²) and averaged. VEGF-positive cells were recognized by brown or yellow-brown granules in the cytoplasm or on the cell membrane. The mean counts of positive and negative cells within three hot spots with the most intensive area of positive cells were calculated to determine the average positive rate of VEGF expression. According to the classification criteria developed by Liu et al., [13] VEGF expression was classified into the following four grades: grade I, < 25 %; grade II, 25-50 %; grade III, 50-75 %; and grade IV, > 75 %.

**Microvascular ultrastructure**

The surgical specimens containing tumor tissue were sliced into 1-mm cubes and fixed in 2.5 % glutaraldehyde with cetylpyridinium buffer, postfixed in 1 % osmium tetroxide, dehydrated, and embedded in Epon. Samples were then sliced with an ultramicrotome to a thickness of 70 nm for transmission electronic microscopy (TEM). The microvascular ultrastructure, including the lumens, endothelial cells, intercellular spaces, basal lamina and extravascular spaces, and other regions were also recorded [14-17].

**Statistical analysis**

The correlation between MVD, VEGF expression, microvascular ultrastructure and the enhanced characteristics of DCE-CT in adrenal masses was evaluated. The quantification data, including the types of TD curves and VEGF expression between the groups, were analyzed using the Pearson χ² test. The measurement data, including MVD and the enhancement washout rates between groups, were analyzed using a two-sample average t-test. If a non-normal distribution was present and remained after numeric conversion, then the Mann-Whitney rank sum application test was used. A p-value less than 0.05 was considered to indicate a statistically significant difference. In addition, receiver-operating characteristic (ROC) curves were used to assess the ability of the washout rate at each delay time to differentiate adenomas from nonadenomas. The sensitivity, specificity, accuracy, positive predictive value and negative predictive value for the diagnosis of adrenal adenomas were all calculated.

**Results**

**TD curves**

The types of TD curves in 45 adrenal masses were as follows: Type A (n = 26), Type B (n = 14), Type C (n = 2), Type D (n = 2), and Type E (n = 1). The types of TD curves were classified into two groups, rapid washout and slow washout. Types A and C were characterized by rapid washout of contrast material, whereas Types B, D and E were characterized by slow washout. Table 1 showed a significant difference in the types of TD curves between adenomas and nonadenomas (P < 0.001). Types A and C were predominantly found in adenomas (Fig. 1 A-C); in comparison, Types B, D, and E were found in nonadenomas (Fig. 2 A-C).
Figure 1  Right lipid-poor adrenal adenoma. (A-C) DCE-CT showed rapid washout with TD curve of Type A, Washr of 34 %, Washa of 53 % at 7-min delay time point; (D) MVD immunohistochemistry (× 200), numerous blood vessels with round lumens, showing higher MVD; (E) VEGF immunohistochemistry (× 200), high positive rate of cells with brown or yellow-brown granules in the cytoplasm, showing high VEGF expression; (F) Microvascular ultrastructure. Transmission electron microscopy (TEM) (× 5000) demonstrated a regular lumen without significant stenosis, a smooth luminal surface, the presence of an excess of pinocytotic vesicles and fenestrations of endothelial cells (thin arrow), a uniform thinning of the basement membrane (empty arrow), a regular and uniform thinning of most of the extravascular space, and the presence of less stroma.

Figure 2  Right adrenal pheochromacytoma. (A-C) DCE-CT showed the slow washout with TD curve of Type B, Washr of 15%, Washa of 49% at 7-min delay timepoint; (D) MVD. Immunohistochemistry (× 200), fewer blood vessels with round lumens, showing lower MVD; (E) VEGF. Immunohistochemistry (× 200), low positive rate of cells with brown or yellow-brown granules in the cytoplasm, showing low VEGF expression; (F) Microvascular ultrastructure. Transmission electron microscopy (TEM) (× 5000) exhibited moderate to severe luminal stenosis or even near occlusion with a small sponge-like appearance, a rough luminal surface, a widening of the intercellular space, a markedly heterogeneous thickening of the basement membrane with prominent discontinuity or disappearances (empty arrow), a significantly irregular and widened extravascular space with plasma-like substances, stromal cells, and a large number of collagen fibers (double arrows).
Twenty-eight adrenal adenomas were diagnosed on the basis of the diagnostic criteria mentioned above, compared with 17 nonadenomas, as shown in Table 2. The sensitivity, specificity and accuracy for the diagnosis of the adenomas were 93%, 94% and 93%, respectively. The positive and negative predictive values were 96% and 89%, respectively.

Table 1  Comparison of the types of TD curves in DCE-CT between adrenal adenomas and nonadenomas

| Adrenal lesions | n | Rapid washout | Slow washout | $\chi^2$ | P value |
|-----------------|---|---------------|--------------|---------|--------|
|                 |   | A + C (n)     | B + D + E (n) |         |        |
| Adenomas        | 27| 26            | 1            | 33.34   | <0.001 |
| Nonadenomas     | 18| 2             | 16           |         |        |

Table 2  Correlation between DET-CT findings and MVD and VEGF in adrenal masses

| Items           | Adrenal masses (n) | MVD (number/0.74mm$^2$) | VEGF [masses (n)] |
|-----------------|--------------------|-------------------------|-------------------|
|                 |                    |                         | Grade II | Grade III | Grade IV |
| Type of TD curve|                    |                         |          |           |           |
| A + C           | 28                 | 70.5 ± 25.2             | 1        | 24        | 3         |
| B + D + E       | 17                 | 39.0 ± 16.4             | 9        | 5         | 3         |
| Washr (%)       |                    |                         |          |           |           |
| $\geq$34        | 28                 | 66.9 ± 24.1             | 3        | 22        | 3         |
| $<34$           | 17                 | 44.9 ± 26.4             | 7        | 7         | 3         |
| Washa (%)       |                    |                         |          |           |           |
| $\geq$43        | 32                 | 66.0 ± 26.3             | 4        | 25        | 3         |
| $<43$           | 13                 | 40.3 ± 19.0             | 6        | 4         | 3         |

Note: Comparison between the rapid washout group and slow washout group of the TD curve, MVD: $t = 4.574, P < 0.001$; VEGF: $\chi^2 = 14.917, P < 0.001$. Comparison between Washr $\geq$34% and $<34%$; MVD: $t = 2.793, P = 0.009$; VEGF: $\chi^2 = 5.679, P = 0.017$. Comparison between Washa $\geq$43% and $<43%$, MVD: $t = 3.645, P = 0.001$; VEGF: $\chi^2 = 6.058, P = 0.014$.

**Washout rate**

ROC curves at the 3-, 5-, and 7-min delay time points were all higher and to the left of the initial curve. Furthermore, the area under the 7-min delay time point curve was highest among the delay time points, which had the largest diagnostic value. Table 3 provides the significant differences in the Washr and Washa at the 7-min delay time point between adenomas and nonadenomas ($P < 0.001$). Moreover, Washr and Washa were significantly higher for adenomas than for nonadenomas.

The optimal threshold values of the washout rate of the 7-min delay time using DCE-CT were identified statistically using ROC curves. The optimal threshold value of Washr for the diagnosis of adenomas and nonadenomas was 34%, in which 28 and 17 adrenal masses were characterized as adenomas (Fig. 1 A-C) and nonadenomas (Fig. 2 A-C), respectively, as shown in Table 2, with a corresponding diagnostic sensitivity, specificity and accuracy of 93%, 94% and 93% for the adenomas, respectively. The positive and negative predictive values were 96% and 89%, respectively. In contrast, the optimal threshold value of Washa was 43%, in which 32 adrenal masses were detected as adenomas, and 13 were classified as nonadenomas on the basis of Table 2, with a corresponding diagnostic sensitivity of 81%, specificity of 92% and accuracy of 84%. The positive and negative predictive values were 96% and 67%, respectively.

**Correlation between DCE-CT findings and angiogenesis in adrenal masses**

MVD and VEGF expression present in rapid washout (Types A and C), Washr $\geq$34% and Washa $\geq$43% at the 7-min delay timepoint were all markedly higher compared to slow washout (Types B, D and E), which
was < 34 % and < 43 %, respectively \((P < 0.05)\) (Table 4; Fig. 1 D, E, Fig. 2 D, E).

**Microvascular ultrastructure**

The microvascular ultrastructure of adrenal adenoma was shown in Table 4, which includes the following features: a regular lumen without significant stenosis, the presence of red blood cells (RBCs) within the lumen, a smooth luminal surface, the swelling of some of the endothelial cells, the presence of an excess of pinocytotic vesicles and fenestrations of endothelial cells, a slight widening of the intercellular gaps, a uniform thinning of the basement membrane with a few breakdowns, the absence of pericytes surrounding the endothelium, a regular and uniform thinning of most of the extravascular space, and the presence of less stroma (Fig. 1 F). In comparison, the microvascular ultrastructure of nonadenomas exhibited the following characteristics: moderate to severe luminal stenosis or even near occlusion with a small sponge-like appearance, the presence of RBCs in the lumen, a rough luminal surface, the presence of endothelial cell proliferation with a decreased number of pinocytotic vesicles and fenestrations, the presence of an excess of pseudopodia, a widening of the intercellular space, a markedly heterogeneous thickening of the basement membrane with prominent discontinuity or disappearances, an excess of surrounding pericytes, a significantly irregular and widened extravascular space with plasma-like substances, stromal cells, and a large number of collagen fibers and a few migrating RBCs (Fig. 2 F).

| Table 3 | Comparison of the washout rate of the 7-min delay timepoint in DCE-CT between adenomas and nonadenomas |
|---------|-----------------------------------------------------------------------------------------------------------------|
| Adrenal masses | Adenomas | Nonadenomas | t value | P value |
| Washr (%) | 49.0 ± 24.5 | 17.5 ± 20.5 | 4.658 | <0.001 |
| Washa (%) | 57.3 ± 10.8 | 20.5 ± 47.2 | 2.916 | <0.001 |

| Table 4 | The microvascular ultrastructure in the rapid washout (Types A and C), Washr ≥ 34%, and Washa ≥ 43% 7 - min delay timepoint was significantly different from the slow washout (Types B, D and E), < 34 %, and < 43 % |
|---------|-----------------------------------------------------------------------------------------------------------------|
| Type of TD curve | A + C | B + D + E |
| Washr (%) | ≥ 34 | < 34 |
| Washa (%) | ≥ 43 | < 43 |

**Microvascular Ultrastructure**

| Lumen | Regular nonstenosis | Irregular stenosis |
|-------|---------------------|--------------------|
| Endothelium | More pinocytotic vesicles and fenestrations | Less pinocytotic vesicles and fenestrations |
| Intercellular space | Widening | Widening |
| Basement membrane | Uniform thinning | Markedly irregular thickening poorer integrity |
| Extravascular space | Regular and uniform thinning, less stroma | Rather irregular and widened, much stroma |

**Discussion**

Dedicated adrenal-protocol CT using a multiphasic examination has been established as an important method for differentiating adrenal adenomas from nonadenomas [10]. However, in our present study, we investigated the correlation between the enhanced characteristics (imaging biomarkers) and histological biomarkers of tumor angiogenesis for the assessment of DCE-CT mechanism in adrenal masses.

The washout curve (TD curve) and washout rate are useful for the diagnosis of adrenal masses. Our present study demonstrated that the DCE-CT findings could be classified into two groups, the rapid washout group and the slow washout group. Types A and C of the TD curve, Washr ≥ 34 %, and Washa ≥ 43 % were characteristic of the rapid washout group; in contrast, types B, D and E, Washr < 34 %, and Washa < 43 % were characteristic of the slow washout group. These results demonstrated that adrenal adenomas were significantly different from nonadenomas with respect to the enhanced features. The washout of contrast material after peak enhancement in adenomas was significantly faster compared to nonadenomas. However, the TD curve for the diagnosis of adenoma was more accurate compared with the washout rate. Thus, we proposed that TD curves should be recommended for its higher diagnostic accuracy,
simplicity and visualization, and if necessary, combined with the washout rate for increasing accuracy.

The correlations between the findings of medical imaging and angiogenesis in tumors have been reported in many studies, in which the interpretation of the enhancement mechanism of medical imaging was based [18-24]. Nevertheless, the enhancement mechanism of DCE-CT/MRI in adrenal masses has not yet been deeply investigated. Thus, the correlation between the characteristics of DCE-CT in adrenal masses and tumor angiogenesis was preliminarily assessed in our present study for the enhancement mechanism.

The correlations between the findings of DCE-CT and MVD in specific tumors have been reported [18-24]. The results of our present study were consistent with previous findings. These results were significantly different in MVD between the rapid washout group (Types A and C of the TD curve, Washr ≥ 34 %, and Washa ≥ 43 %) and the slow washout group (Types B, D and E, Washr < 34 %, and Washa < 43 %). The MVD in the rapid washout group was significantly higher than in the slow washout group. Thus, this finding indicated that the MVD in the adrenal mass is one of the most important factors affecting the distribution of the TD curve type and the difference in the washout rate of DCE-CT. However, there was a significant difference in the distribution of the TD curve type and washout rate between adrenal adenomas and nonadenomas. Types A and C, Washr ≥ 34 %, and Washa ≥ 43 % showed rapid washout after peak enhancement, which are characteristics of adrenal adenomas; in contrast, Types B, D and E, Washr < 34 %, and Washa < 43 % showed slow washout, which were characteristics of nonadenomas. On the basis of our results, the MVD is statistically and significantly higher in adenomas than in nonadenomas, and thus, it could be concluded that MVD is one of most important histopathological factors affecting the ability to differentiate adenomas from nonadenomas. Consequently, a higher VEGF expression in adenomas could be attributed to rapid washout after peak enhancement in DCE-CT, and may also become fundamental for the diagnosis of adrenal adenoma.

Zhou and Reitan, et al. identified a correlation between the findings of DCE-CT and microvascular ultrastructure in specific tumors [25,26]. The results of our present study are consistent with previous results. There were significant differences in the microvascular ultrastructure between the rapid washout group (Types A and C of the TD curve, Washr ≥34 %, and Washa ≥43 %) and the slow washout group (Types B, D and E, Washr <34 %, and Washa <43 %). The characteristics of the microvascular ultrastructure of the adrenal mass in the rapid washout group were significantly different compared with those in the slow washout group. The former showed the following characteristics: a regular lumen without significant stenosis, a smooth luminal surface, an excess of pinocytotic vesicles and fenestrations of endothelial cells, a slight widening of the intercellular spaces, a uniform thinning of the basement membrane with a few breakdowns, the absence of pericytes surrounding the endothelium, a regular and uniform thinning of most extravascular spaces, and the presence of less stroma. However, the latter showed the following characteristics: moderate to severe luminal irregular stenosis, a rough luminal surface, endothelial cell proliferation with a decreased number of pinocytotic vesicles and fenestrations, an excess of pseudopodia, widening of the intercellular space, markedly heterogeneous thickening of the basement membrane with prominent discontinuity or disappearances, an excess of surrounding pericytes, significantly irregular and widened extravascular space with plasma-like substances, the presence of stromal cells, and a large number of collagen fibers. Thus, this comparison revealed that the microvascular ultrastructure in the adrenal mass was also one of the most important factors that affected the enhanced features in DCE-
CT. As previously described above, there was also a significant difference in the enhanced features between adrenal adenomas and nonadenomas. Rapid washout was characterized by adrenal adenomas in DCE-CT. In contrast, slow washout was characterized by adrenal nonadenomas. According to our results, the findings of the microvascular ultrastructure in adenomas were the same in rapid washout after peak enhancement; in contrast, appearances of the microvascular ultrastructure in nonadenomas were observed in the slow washout condition. It could be assumed that the microvascular ultrastructure is one of the most important histopathological factors for distinguishing adenomas from nonadenomas. Thus, the regular microvascular ultrastructure in the adenoma could result in rapid washout after peak enhancement in DCE-CT, making this technique valuable for the diagnosis of adrenal adenoma.

Our present study had several limitations. First, the number of adrenal metastases was relatively small compared with the number of adenomas. Second, most patients were referred to the dedicated CT protocol because they demonstrated having adrenal masses that were discovered incidentally and because they had evidence of hyperfunctioning adrenal masses. Finally, this study was only performed in our own institution, and thus, a multicenter study is required for further confirmation of the diagnostic value and enhancement mechanism of DCE-CT for the differentiation of adenomas from nonadenomas.

Conclusion

In a summary, this study demonstrated the correlation between the DCE-CT imaging biomarkers and histological biomarkers of tumor angiogenesis in adrenal adenomas and nonadenomas, in which our findings illuminate this enhancement mechanism.

Acknowledgments

This study was supported by grants from project of Shanghai science and Technology Commission of Jiading District City (JDKW-2021-0014).

Conflict of Interest

The authors have no conflict of interest to declare.

References

[1] Koo HJ, Choi HJ, Kim HJ, Kim SO, Cho KS. The value of 15-minute delayed contrast-enhanced CT to differentiate hyperattenuating adrenal masses compared with chemical shift MR imaging. Eur Radiol. 2014; 24: 1410-1420.

[2] Park SW, Kim TN, Yoon JH, Kim TH, Chung JM, Jeon UB, et al. The washout rate on the delayed CT image as a diagnostic tool for adrenal adenoma verified by pathology: a multicenter study. Int Urol Nephrol. 2012; 44: 1397-1402.

[3] Kumagae Y, Fukukura Y, Takumi K, Shindo T, Tateyama A, Kamiyama T, et al. Distinguishing adrenal adenomas from non-adenomas on dynamic enhanced CT: a comparison of 5 and 10 min delays after intravenous contrast medium injection. Clin Radiol. 2013; 68: 696-703.

[4] Sangwaiya MJ, Boland GW, Cronin CG, Blake MA, Halpern EF, Hahn PF. Incidental adrenal lesions: accuracy of characterization with contrast-enhanced washout multidetector CT—10-minute delayed imaging protocol revisited in a large patient cohort. Radiology. 2010; 256: 504-510.

[5] Kamiyama T, Fukukura Y, Yoneyama T, Takumi K, Nakajo M. Distinguishing adrenal adenomas from nonadenomas: combined use of diagnostic parameters of unenhanced and short 5-minute dynamic enhanced CT protocol. Radiology. 2009; 250: 474-481.

[6] Ilias I, Sahdev A, Reznek RH, Grossman AB, Pacak K. The optimal imaging of adrenal tumours: a comparison of different methods. Endocr Relat Cancer. 2007; 14: 587-599.

[7] Blake MA, Kalra MK, Sweeney AT, Lucey BC, Maher MM, Sahani DV, et al. Distinguishing benign from malignant adrenal masses: multi-detector row CT protocol with 10-minute delay. Radiology. 2006; 238: 578-585.

[8] Caoili EM, Korobkin M, Francis IR, Cohan RH, Platt JF, Dunick NR, et al. Adrenal masses: characterization with combined unenhanced and delayed enhanced CT. Radiology. 2002; 222: 629-633.

[9] Pena CS, Boland GW, Hahn PF, Lee MJ, Mueller PR. Characterization of indeterminate (lipid-poor) adrenal masses: use of washout characteristics at contrast-enhanced CT. Radiology. 2000; 217(3): 798-802.

[10] Korobkin M, Broedel FJ, Francis IR, Quint LE, Dunick NR, Londy F. CT time-attenuation washout curves of adrenal adenomas and nonadenomas. AJR Am J Roentgenol. 1998; 170: 747-752.

[11] Szolar DH, Kammerhuber FH. Adrenal adenomas and nonadenomas: assessment of washout at delayed contrast-enhanced CT. Radiology. 1998; 207: 369-375.

[12] Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma. N Engl J Med. 1991; 324: 1-8.

[13] Peifang L, Runxian B, Yun N, Yong Y. Angiogenesis and dynamic contrast enhanced MRI of benign and malignant breast lesions: preliminary results. Chin J Radiol. 2002; 36: 967-972.

[14] Miyagami M, Nakamura S. Ultrastructure of microvessels and tumor organization and structure in experimental brain tumors: microvessel cell processes on central neurocytoma. Brain Tumor Pathol. 1997; 14: 79-83.

[15] Schlageter KE, Molnar P, Lapin GD, Groothuis DR. Microvessel organization and structure in experimental brain tumors: microvessel populations with distinctive structural and functional properties. Microvasc Res. 1999; 58: 312-328.

[16] Caruso A, Efficace F, Parrila A, Angelone L, Ferranti F, Grandinetti ML. Pain and anxiety related to mammography in breast cancer patients. Psychological evaluation in an experimental study. Radiol Med. 2001; 102: 335-339.

[17] Caruso RA, Bonanno A, Finocchiaro G, Cavaleri R, Gatto G, Platino FM, et al. Ultrastructural observations on inflammatory angiogenesis in gastric carcinomas with massive neutrophil infiltration. Ultrastruct Pathol. 2009; 33: 1-5.

[18] Chen Y, Zhang J, Dai J, Feng X, Lu H, Zhou C. Angiogenesis of renal cell carcinoma: perfusion CT findings. Abdom Imaging. 2010; 35: 622-628.

[19] Bai RJ, Cheng XG, Qu H, Shen BZ, Han MJ, Wu ZH. Solitary pulmonary nodules: comparison of multi-slice computed tomography...
perfusion study with vascular endothelial growth factor and microvessel density. *Chin Med J (Engl).* 2009; 122: 541-7.

[20] Jiang NC, Han P, Zhou CK, Zheng JL, Shi HS, Xiao J. Dynamic enhancement patterns of solitary pulmonary nodules at multi-detector row CT and correlation with vascular endothelial growth factor and microvessel density. *Ai Zheng.* 2009; 28: 164-169.

[21] Chen WX, Min PQ, Song B, Xiao BL, Liu Y, Ge YH. Single-level dynamic spiral CT of hepatocellular carcinoma: correlation between imaging features and density of tumor microvessels. *World J Gastroenterol.* 2004; 10: 67-72.

[22] Tateishi U, Kusumoto M, Nishihara H, Nagashima K, Morikawa T, Moriyama N. Contrast-enhanced dynamic computed tomography for the evaluation of tumor angiogenesis in patients with lung carcinoma. *Cancer.* 2002; 95: 835-842.

[23] Qin HY, Sun H, Wang X, Bai R, Li Y, Zhao J. Correlation between CT perfusion parameters and microvessel density and vascular endothelial growth factor in adrenal tumors. *PLoS One.* 2013; 8: e79911.

[24] Tateishi U, Nishihara H, Watanabe S, Morikawa T, Abe K, Miyasaka K. Tumor angiogenesis and dynamic CT in lung adenocarcinoma: radiologic-pathologic correlation. *J Comput Assist Tomogr.* 2001; 25: 23-27.

[25] Zhou H, Liu JK, Chen SX, Xiong Z, Lin GQ, Zhou ML, et al. Correlation of blood flow assessed by CT perfusion imaging and microvascular ultrastructure in non-small cell lung cancer: a preliminary study. *Zhong Hua Zhong Liu Za Zhi.* 2013; 35: 193-7. [In Chinese].

[26] Reitan NK, Thuen M, Goa PE, de Lange Davies C. Characterization of tumor microvascular structure and permeability: comparison between magnetic resonance imaging and intravital confocal imaging. *J Biomed Opt.* 2010; 15: 036004.