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Host-related Risk Factors for Adherent Perinephric Fat in Healthy Individuals Undergoing Laparoscopic Living-donor Nephrectomy

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Purpose: The purpose of this study is to assess the risk factors and characteristics of adherent perinephric fat (APF) in healthy individuals.

Patients and Methods: Men who underwent laparoscopic donor nephrectomy were included. Video review was used to divide patients on the basis of APF severity. Relationship between APF scores and clinical and radiographic features was evaluated.

Results: Of the 92 patients, 43 (46.7%) and 8 (8.7%) were categorized as APF and severe APF, respectively. The median total operative time was significantly associated with APF severity. Sex, body mass index, and perinephric fat area, stranding, and thickness were significantly associated with severe APF. In the multivariate analysis, perinephric fat areas and stranding were independent risk factors for severe APF (HR, 1.189 and 14.450, respectively). In the 44 analyzed cytokines, levels of sIL-6R in the perinephric adipose tissue-conditioned medium were significantly higher for APF group than that for non-APF group ($P = 0.049$).

Conclusions: Host-related risk factors for APF could predict surgical difficulty in patients undergoing partial nephrectomy.

Key Words: adherent perinephric fat, IL-6R, living donor, nephrectomy, renal surgery, sticky fat

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Partial nephrectomy is a standard procedure when managing a clinical T1a renal mass, although its indications have been expanded to include larger renal tumors.[1] In recent years, surgeons have increasingly relied on renal morphometry scores to predict surgical difficulties of nephron-sparing surgery, including the RENAL nephrometry score, the PADUA prediction score, and the C-index.[2] Although these tumor-related factors help to predict surgical difficulties and avoid surgical complications, patient-specific factors related to surgical complexities may also be critical to completing partial nephrectomy without complication.

A potential host-related risk factor for surgical difficulty is the presence of thick and adherent adipose tissue surrounding the kidney; this has been called adherent perinephric fat (APF) or sticky fat.[2,3] Recent studies have since established the incidence and/or predictive factors for APF[2,3] and proposed it as a predictor of surgical outcomes.[4] Although APF is a host-related factor, all subjects in previous studies had renal tumors. Therefore, the true host-related risk factors for APF are not known for patients who are tumor free.

In this study, we evaluated the incidence and effects of APF on surgical outcomes and risk factors for APF in healthy individuals treated by living-donor nephrectomy. We also assessed the characteristics of perinephric adipose tissue (PAT) in patients with APF to clarify its importance as a pure host-related risk factor for surgical difficulties during kidney dissection.

PATIENTS AND METHODS

Patients and Surgical Procedure

The medical records of 92 donors who underwent laparoscopic donor nephrectomy (LDN) at Akita University School of Medicine between 2009 and 2014 were retrospectively reviewed after Institutional Review Board approval. Among the LDN procedures, 21 donors underwent a standard LDN (June 2009 to October 2010), 29 underwent a single-site plus 1 trocar LDN (November 2010 to November 2011), and 42 underwent a pure single-port LDN (December 2011 to January 2013). Right-sided LDN was excluded from this study because of the small sample and to avoid location bias on surgical outcomes. All procedures were performed by 11 senior urologists experienced in urological laparoscopic surgery. The donor eligibility,

operative procedures, and surgical outcomes at our institution have been described previously.[5–7]

Data Collection

Patient characteristics such as age, body mass index (BMI), sex, preoperative serum creatinine, preoperative estimated glomerular filtration rate, and smoking status were collected from medical records, together with the details of operative variables such as operative time and estimated blood loss. The severity of APF was categorized into 3 groups as non-APF, APF, and severe APF on the basis of retrospective review of the video-recorded surgical procedures during anterior perinephric fat dissection, which were routinely performed during our donor nephrectomy to minimize the length of the wound as previously reported.[8] Non-APF was defined as easily-dissected, nonadherent perinephric fat. In contrast, severe APF was defined as perinephric fat requiring exfoliation of the renal capsule for exposure of the renal parenchyma, or as fat remaining on the parenchymal surface because of difficulty when dissecting the anterior perinephric fat. APF was defined as mildly adherent without subcapsular dissection, which was in between the criteria for non-APF and severe APF. These definitions of APF status were modified by referring to previous studies.[2,3]

Imaging Analyses

The perinephric fat status on the preoperative computed tomography (CT) was measured at the level of the renal vein, with the reviewer blinded to the APF status on video examination. The methods for measuring the visceral fat and perinephric fat area have previously been described.[6] The thickness and area of fat were measured using a Synapse Vincent volume image analyzer system (Fujifilm, Tokyo, Japan). The presence of perinephric stranding, which was defined as a linear area of soft tissue attenuation in the perinephric space,[2] was also assessed by CT.

Macrophage Infiltration in PAT

The research using human adipose tissues were initiated after receiving institutional review board approval in Akita University Graduate School of Medicine. PAT harvested from donors was fixed, embedded in paraffin, and prepared in 5-μm sections. Immunohistochemical staining was performed with primary antibody against CD68 (1:100, Dako, Glostrup, Denmark). Immunostaining of tissue sections was done as previously described.[9] Macrophages were counted within a 200-μm-diameter circle in 3 random fields, as previously described.[10] A pathologist who was blinded to the APF results evaluated the morphologic and immunostaining findings and counted cell numbers of the positive staining.

Cytokine Measurements

PAT was harvested during surgery, and tissue procurement and conditioned medium generation followed the previously reported technique.[11] Briefly, fat tissue was transferred to a Petri dish containing 20 mL of phosphate-buffered saline (PBS) and finely minced using scissors into pieces weighing 20 to 80 mg. Tissue pieces were extensively washed with 200 mL PBS over a 70-μm pore-size filter (BD Biosciences) and transferred to a 50-mL centrifuge tube containing 45 mL of PBS at 37°C, which was then gently shaken for 20 minutes. The tube contents were poured over the filter and the tissue pieces were transferred to a tube containing 50 mL of PBS and centrifuged for 1 minute at 277× gravity at room temperature to remove red blood cells and debris. The fat tissue was placed in a Petri dish with 10 mL of M199 culture medium (Invitrogen) supplemented with 50 μg/mL gentamicin per gram of tissue. Medium collected after 24 hours was aliquoted into 1-mL fractions and stored at −80°C. Multiplex analysis of perinephric fat tissue-condition medium was conducted using a SearchLight Multiplex assay (Aushon Biosystem Inc., Billerica, MA) to screen for the increased levels of a panel of proteins, including 44 cytokines. The levels of soluble interleukin-6 receptor (sIL-6R) in PAT were validated by enzyme-linked immunosorbent assay, using a human sIL-6R immunoassay in independent patients evaluated by multiplex analysis (Quantikine, Funakoshi).

Statistical Analysis

Two group comparisons were performed by the Mann-Whitney *U* test or the *t* test for continuous variables. Statistical differences for trend among 3 groups were assessed using the Jonckheere-Terpstra test.[12] The association of clinical variables with the presence of severe APF was evaluated by logistic regression models with odds ratios (ORs) and 95% confidence intervals (95% CIs). To be included in the multivariate analysis, variables were required to be significant in the univariate analysis. All statistical analyses were performed using the SPSS software package, version 19.0 (SPSS Inc., Chicago, IL). All reported *P*-values were 2 sided, and statistical significance was considered at $P < 0.05$.

RESULTS

The baseline characteristics of all patients are summarized in Table 1. Of the 92 patients, 41 (44.6%), 43 (46.7%), and 8 (8.7%) were categorized into the non-APF group, the APF group, and the severe APF group, respectively. Thirty-six patients (39.1%) were men, and the median age was

TABLE 1. Patient Characteristics

Variables	N = 92 [n (%)/ Median (Range)]
Sticky fat status	
Non-APF	41 (44.6)
APF	43 (46.7)
Severe APF	8 (8.7)
Sex	
Male	36 (39.1)
Female	56 (60.9)
History of smoking	
No	74 (80.4)
Yes	18 (19.6)
Age (y)	59.5 (25-79)
BMI (kg/m^2)	23.5 (16.3-38.5)
Baseline creatinine (mg/dL)	0.60 (0.39-0.96)
Baseline eGFR (mL/min/1.73 m^2)	89.2 (49.6-139.3)
Visceral fat area (cm^2)	60.5 (0.3-233.7)
Perinephric fat area (cm^2)	9.1 (0.04-58.2)
Posterior perinephric fat thickness (cm)	0.8 (0.09-2.3)
Lateral perinephric fat thickness (cm)	1.0 (0.1-3.4)
Renal stranding	
No	86 (93.5)
Yes	6 (6.5)

APF indicates adherent perinephric fat; BMI, body mass index; eGFR, estimated glomerular filtration rate.

59.5 years. The median BMI was 23.5 kg/m^2, including 60 patients (65.2%) of normal weight, 25 (27.2%) who were overweight, and 7 (7.6%) who were obese, according to the World Health Organization classification. The median preoperative serum creatinine level was 0.60 mg/dL (range, 0.39 to 0.96 mg/dL), and median estimated glomerular filtration rate was 89.2 mL/min/1.73 m^2 (range, 49.6 to 139.3 mL/min/1.73 m^2). Because all patients were candidates for living-donor transplantation, they had no underlying diseases, including cardiovascular disease, diabetes mellitus, or severe hypertension. However, 18 (19.6%) were regular smokers.

The median values for the visceral fat area, perinephric fat area, posterior perinephric fat thickness, and lateral perinephric fat thickness were 60.5 cm^2 (range, 0.3 to 233.7 cm^2), 9.1 cm^2 (range, 0.04 to 58.2 cm^2), 0.8 cm (range, 0.09 to 2.34 cm), and 1.0 cm (range, 0.14 to 3.57 cm), respectively. Renal stranding was observed on the CT scans of 6 patients (6.5%). Regarding the operative outcomes, the median operative time was 234 minutes (range, 136 to 342 min), and the median estimated blood loss was 25 mL (range, 0 to 347 mL).

First, we assessed the relationship between surgical outcomes and perinephric fat status. The mean operative time was significantly associated with perinephric fat status (trend, $P = 0.00248$; Fig. 1), and the operative times in both the APF and severe APF were significantly longer than that in the non-APF group ($P = 0.001$, 0.042, respectively; Fig. 1). In addition, patients with severe APF had a significantly higher estimated blood loss than patients in the non-APF group ($P = 0.002$). These results suggest that the perinephric fat status influenced the surgical outcomes during LDN.

Next, we explored the risk factors for severe APF. In the univariate analysis (Table 2), there was a significantly increased likelihood of severe APF in the following groups: those with a higher BMI (OR, 1.213; $P = 0.013$), men (OR, 5.400; $P = 0.047$), those with a greater visceral fat area (OR, 1.027; $P < 0.001$), those with a greater perinephric fat area (OR, 1.134; $P < 0.001$), those with longer posterior perinephric fat thickness (OR, 19.579; $P = 0.001$), those with longer lateral perinephric fat thickness (OR, 4.061; $P = 0.006$), and those with renal stranding (OR, 16.200; $P = 0.003$) (Table 2). In particular, mean perinephric fat area was significantly associated with the likelihood of APF (7.5 cm^2, 12.5 cm^2, and 31.7 cm^2 in the non-APF, APF, and severe APF groups, respectively; trend; $P < 0.001$, supplementary Fig. 1, Supplemental Digital Content 1, http://links.lww.com/SLE/A161). The other perioperative parameters were not significantly different among the 3 groups. As shown in Table 2, the perinephric fat area (OR, 1.189; $P = 0.005$) and the presence of renal stranding (OR, 14.450; $P = 0.037$) were independent risk factors for the presence of severe APF.

To evaluate the characteristics of APF, we examined macrophage infiltrations in PAT between the groups with and without APF (including severe APF); however, there were no significant differences in the mean number of CD68$^+$ cells between the 2 groups (4.74 cells/200 μm^2 compared with 4.83 cells/200 μm^2; $P = 0.934$, supplementary Fig. 2, Supplemental Digital Content 2, http://links.lww.com/SLE/A162). To screen for secreted cytokines from PAT, we performed multiplex assays of 44 cytokines from adipose condition medium in 10 individuals (7 without APF and 3 with APF). In this analysis, the mean levels of sIL-6R in the PAT-conditioned medium were significantly higher in the group with than without APF ($P = 0.049$), although there was no statistically significant difference in the mean level of other cytokines between the 2 groups (Table 1, Supplemental Digital Content 3, http://links.lww.com/SLE/A163). The difference in the levels of sIL-6R in the PAT condition medium was then validated in a larger number of donors (n = 48), in which the mean sIL-6R levels remained significantly higher in both groups with APF (n = 32; 223.7 pg/μL) when compared with the non-APF group (n = 16; 145.7 pg/μL) ($P = 0.042$; Fig. 2).

DISCUSSION

In this study, we showed that a larger perinephric fat area and the presence of renal stranding on preoperative CT were independent risk factors for severe APF in healthy

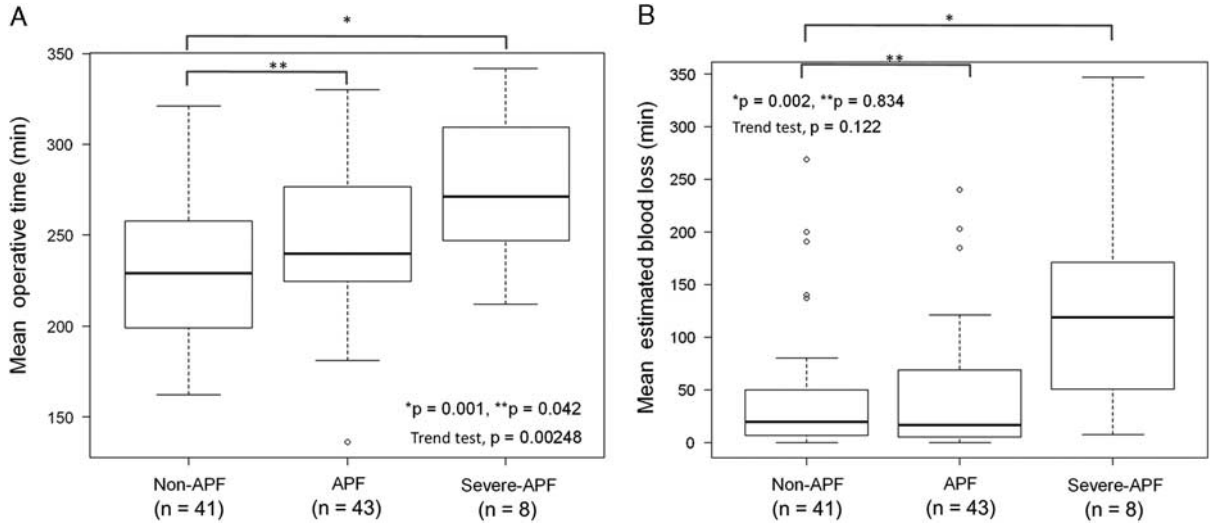

FIGURE 1. The association between surgical outcomes and perinephric fat status. Operative time (A) and estimated blood loss (B) were compared among 3 groups by the presence and severity of (APF): non-APF (n = 41), APF (n = 43), and severe APF (n = 8). The results are presented as box and whisker plots with median (interquartile range). APF indicates adherent perinephric fat.

TABLE 2. Univariate and Multivariate Proportional Hazard Regression Model Predicting Perirenal Severe Sticky Fat in Transplant Living Donor

	Univariate			Multivariate (Stepwise)		
	Hazard Ratio	**95% CI**	***P***	**Hazard Ratio**	**95% CI**	***P***
Age (y)	1.074	0.987-1.169	0.096	—	—	—
BMI (kg/m^2)	1.213	1.042-1.141	0.013	—	—	—
Sex male (vs. female)	5.400	1.025-28.439	0.047	—	—	—
History of smoking (vs. No)	1.776	0.204-15.429	0.603	—	—	—
Baseline serum creatinine (mg/dL)	145.979	0.817-26,072.226	0.060	—	—	—
Baseline eGFR (mL/min/1.73 m^2)	0.978	0.936-1.022	0.321	—	—	—
Visceral fat area (cm^2)	1.027	1.012-1.041	< 0.001	—	—	—
Perinephric fat area (cm^2)	1.134	1.059-1.215	< 0.001	1.189	1.053-1.343	0.005
Posterior perinephric fat thickness (cm)	19.579	3.535-108.445	0.001	—	—	—
Lateral perinephric fat thickness (cm)	4.061	1.485-11.103	0.006	—	—	—
Renal stranding yes (vs. no)	16.200	2.579-101.757	0.003	14.450	1.181-176.844	0.037

BMI indicates body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate.

individuals undergoing living-donor nephrectomy. In addition, the secretion of sIL-6R from PAT in individuals with APF was significantly higher than that in those without APF. This is a first report to clarify these host-related risk factors and the functional aspect of APF during nephrectomy, without the confounding influence of tumor-related factors.

The incidence of APF in patients with renal cell carcinoma undergoing partial nephrectomy has been reported at 30% to 55.2%.[2–4] An influential factor in the differences in frequency among the previous studies was the use of different definitions of APF. Bylund et al[3] used their medical records to define the severity of APF, whereas Zheng et al[4] divided APF severity into 3 groups using the duration of fat dissection. Although our definition of severe APF was comparable to that used in the study by Davidiuk and colleagues, the rate was markedly lower in our study (8.7% compared with 30%). An explanation for the lower APF rate in this study was the absence of renal cell carcinoma. Bylund et al[3] revealed that tumor-related risk factors, including tumor size and tumor >50% exophytic, were significantly associated with perinephric sticky fat. In another study, the percentage of APF was shown to increase with tumor size.[2] Thus, to some extent, tumor-related factors appear to influence the presence and severity of APF. Furthermore, high BMI has been strongly associated with the presence of APF in previous studies,[2] and our univariate findings were consistent. The low numbers of overweight and obese individuals in our Japanese population may also have resulted in the lower rate of APF when compared with other studies.

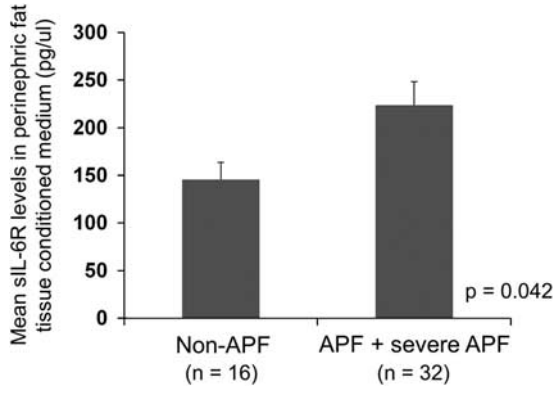

FIGURE 2. Comparison of sIL-6R levels in perinephric fat tissue condition medium by the presence of adherent perinephric fat. The bar graph represents the mean and standard error values for each group. Statistical significance was indicated by *P*-values <0.05. APF indicates adherent perinephric fat.

Perinephric fat status on preoperative CT is one of the most important factors to consider when predicting the presence of APF.[2,4] When assessing perinephric fat status, the posterior perinephric fat thickness has previously been shown to be significantly associated with the presence of APF.[3] In this study, we tried to assess the impact, not only of perinephric fat thickness, but also of the perinephric fat area, which is expected to be a more accurate measurement of perinephric fat volume in the presence of severe APF. However, the posterior perinephric fat thickness was not an independent prognostic factor of the presence of severe APF, despite being significantly associated with the presence of severe APF in the univariate analysis. In contrast, the presence of a perinephric fat area was an independent predictor of severe APF in the multivariate analysis. The results suggest that more accurate measurement of perinephric fat volume has a larger impact on predicting the presence of APF.

Another candidate prognostic factor for the presence of severe APF was the density of perinephric fat on CT scan. Davidiuk et al[2] categorized their participants into 3 groups on the basis of the severity of renal stranding, showing that each group had a different OR for APF. They proposed a scoring system on the basis of the categorized renal stranding status to predict the presence of APF.[2] In contrast to their approach, we simply divided patients into 2 groups on the basis of the presence or absence of perinephric fat stranding, because we only had 6 patients with mild or severe renal stranding. In other research, Zheng et al[4] assessed the impact of perinephric fat surface density and showed it to be the only independent risk factor of difficulty during perinephric fat dissection among patients who underwent partial nephrectomy. Further study is warranted to investigate the most useful classification of APF density when predicting APF.

The impact of the presence of APF on clinical outcomes following partial nephrectomy is still controversial. In the study of patients treated with partial nephrectomy and laparoscopic cryoablation, the median total operative time in patients with sticky fat was nearly 40 minutes longer than that in the control group.[3] In contrast, Davidiuk

et al[13] showed that APF was not statistically associated with an increased likelihood of surgical complications in patients who underwent robot-assisted laparoscopic partial nephrectomy. Although we showed that the severe APF group had a significantly longer operative time and higher estimated blood loss when compared with the non-APF group, we could not evaluate the relationship by correlation analyses, adjusting for other factors such as BMI, age, and previous surgery. Therefore, the impact of APF on clinical outcomes in this population should be investigated in a future study.

The presence of the APF-related cytokine, sIL-6R, was confirmed in this study. The soluble form of the IL-6 receptor has been detected in the serum, urine, and culture media of IL-6R-positive human cells.[14] A recent study reported that obese individuals had higher levels of IL-6R expression in their adipose tissue compared with lean or overweight subjects.[15] Furthermore, in a study among women, the median level of serum sIL-6R was significantly higher in obese compared with normal-weight groups.[16] Although the correlation between local and serum expressions of sIL-6R remains largely unknown, the presence of elevated serum sIL-6R may represent a potential biomarker for the presence of APF. In addition, further study is warranted to determine the detailed mechanisms surrounding the functional role of sIL-6R on APF.

This study has several limitations. First, the small sample size and retrospective nature might have introduced bias. Second, although we used only 1 researcher (blinded) for APF categorization to avoid interresearcher differences, the video-based categorization was inherently subjective. Lastly, the functional roles of cytokines secreted from APF remain unclear, and will need to be clarified in future studies.

In conclusion, in this study, we successfully assessed the host-related risk factors for APF in cancer-free, healthy individuals undergoing nephrectomy for kidney transplantation. Several radiographic features, including the presence of a perinephric fat area or the presence of perinephric fat stranding, were shown to be risk factors for severe APF. Moreover, we found that sIL-6R secreted from adipose tissues might be associated with APF in these patients. We therefore conclude that host-related risk factors for APF could be useful when predicting surgical difficulty in patients undergoing partial nephrectomy, and that the specific cytokine, sIL-6R, has the potential to become a biomarker of APF.

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