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Establishment of Reference Values for Non-HLA Antibodies in Patients With End-stage Renal Disease

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Dear Editor,

Non-HLA expression varies with anatomic location and vessel type. Extensive kidney cell damage and apoptosis in patients with end-stage renal disease (ESRD) trigger the release of intracellular proteins that are inaccessible to antibodies. This reaction boosts autoantibody formation to eliminate these antigens from the circulation [1]. Thus, non-HLA antibody levels may differ between patients with ESRD and healthy individuals. We aimed to establish reference values for patients with ESRD and investigate the factors affecting their non-HLA antibody levels.

This retrospective observational study included non-transplant patients with ESRD aged ≥18 years from January 2015 to July 2020. The institutional review board (IRB) of Pusan National University Yangsan Hospital (Yangsan, Korea) approved the study (05-2021-248). All samples were obtained from the Biobank with informed consent under IRB-approved protocols. We used the single-antigen bead-based LABScreen assay (One Lambda, Canoga Park, CA, USA) following the manufacturer's instructions to determine non-HLA antibody levels. To define positive and negative reactions, we used the manufacturer's reference values, which were calculated using the median of the trimmed mean fluorescence output data plus twice the standard deviation in 95% of the 125 non-transplant patients. We calculated reference values using the same method and our study data. Statistical analyses were performed using the MedCalc Statistical Software version 18.11.3 (MedCalc, Ostend, Belgium). Statistical significance was set at $P<0.05$.

In the 241 patients, the antibody positivity rates based on the manufacturer's and our reference values ranged 0.4%–97.1% and 10.3%–14.9%, respectively (Table 1). More than 50% of the patients tested positive for interferon gamma (IFNG), protein kinase C (PRKCH), regenerating islet-derived protein 3 alpha (REG3A), and vimentin (VM) antibodies based on the manufacturer's reference values. The positivity rates of 22 antibodies calculated based on the manufacturer's and our reference values differed significantly (Table 1).

All patients had one or more antibodies based on the manufacturer's reference values; however, 30 patients lacked the antibodies assessed according to our reference values. Moreover, 186 patients had <10 antibodies (including 47 patients with only one antibody), and 25 patients had ≥10 antibodies (including four patients with >20 antibodies) according to our reference values. Only glutathione S-transferase theta 1 (GSTT1) antibody levels were higher in women than in men (1,513.8 [124.4–15,970]

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Table 1. Positivity rates for 32 non-HLA antibodies based on the manufacturer's reference values (95%) and those established in the present study (N=241)

Antibody target	Manufacturer		Current study		*P*
	Reference values (MFI)	N of positive cases (%)	Reference values (MFI)	N of positive cases (%)	
AGRIN	504	13 (5.3)	308	32 (13.3)	0.004
AGT	1,641	50 (20.7)	2,415	30 (12.4)	0.020
ARHGDIB	3,918	12 (5.0)	1,897	26 (10.8)	0.027
AURKA	4,892	15 (6.2)	3,360	33 (13.6)	0.009
CD36	1,591	11 (4.5)	569	27 (11.2)	0.010
CHAF1B	11,722	6 (2.5)	6,324	30 (12.4)	<0.001
CSCL9	575	29 (12.0)	563	31 (12.8)	0.890
CXCL10	285	73 (30.3)	433	28 (11.6)	<0.001
CXCL11	309	96 (39.8)	574	28 (11.6)	<0.001
EIF2A	6,901	7 (2.9)	3,249	36 (14.9)	<0.001
ENO	4,218	50 (20.7)	5,341	35 (14.5)	0.094
FLRT	688	78 (32.4)	1,225	29 (12.0)	<0.001
GAPDH	508	75 (31.1)	1,345	27 (11.2)	<0.001
GDNF	1,004	14 (5.8)	733	31 (12.8)	0.012
GSTT1	6,136	29 (12.0)	5,025	36 (14.9)	0.424
HNRNPK	845	102 (42.3)	1,578	25 (10.3)	<0.001
IFIH1	3,870	28 (11.6)	3,428	33 (13.6)	0.584
IFNG	498	128 (53.1)	1,090	27 (11.2)	<0.001
LG3	4,154	10 (4.1)	1,900	31 (12.8)	0.001
LMNA	6,633	12 (5.0)	4,111	31 (12.8)	0.002
LMNB	2,065	24 (10.0)	1,804	28 (11.6)	0.660
Myosin	9,341	5 (2.1)	6,619	28 (11.6)	<0.001
PECR	4,120	42 (17.4)	4,743	34 (14.1)	0.382
PLA2R	195	24 (10.0)	180	28 (11.6)	0.660
PPIA	3,292	39 (16.2)	3,657	34 (14.1)	0.612
PRKCH	1,048	151 (62.6)	3,663	27 (11.2)	<0.001
PRKCZ	9,104	30 (12.4)	9,334	27 (11.2)	0.778
PTPRN	3,042	26 (10.8)	2,648	34 (14.1)	0.334
REG3A	86	234 (97.1)	676	27 (11.2)	<0.001
TNFA	5,331	1 (0.4)	932	28 (11.6)	<0.001
TUBA1B	1,987	17 (7.0)	1,318	28 (11.6)	<0.001
VM	820	125 (51.9)	3,092	30 (12.4)	<0.001

Abbreviations: AGRIN, agrin; AGT, angiotensinogen; ARHGDIB, rho GDP dissociation inhibitor 2; AURKA, aurora kinase A-interacting protein; CD36, platelet glycoprotein 4; CHAF1B, chromatin assembly factor 1 subunit B; CXCL10, C-X-C motif chemokine 10; CXCL11, C-X-C motif chemokine 11; CXCL9, C-X-C motif chemokine 9; EIF2A, eukaryotic translation initiation factor 2A; ENO1, α-enolase; FLRT, fibronectin leucine-rich transmembrane protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GDNF, glial cell line-derived neurotrophic factor; GSTT1, glutathione S-transferase theta 1; HNRNPK, heterogeneous nuclear ribonucleoprotein K; IFIH1, interferon-induced helicase C domain-containing protein 1; IFNG, interferon gamma; LG3, basement membrane-specific heparan sulfate proteoglycan core protein; LMNA, prelamin-A/C; LMNB, lamin-B1; myosin, cardiac-type myosin-binding protein C; MFI, median fluorescence intensity; PECR, peroxisomal trans-2-enoyl-CoA reductase; PLA2R, secretory phospholipase A2 receptor; PPIA, peptidyl-prolyl cis-trans isomerase A; PRKCH, protein kinase C eta type; PRKCZ, protein kinase C zeta type; PTPRN, receptor-type tyrosine-protein phosphatase-like N; REG3A, regenerating islet-derived protein 3 alpha; TNFA, tumor necrosis factor alpha; TUBA1B, tubulin alpha 1B chain; VM, vimentin.

median fluorescence intensity (MFI) vs. 816.3 [76.3–12,079.2] MFI). Only GSTT1, IFNG, and secretory phospholipase A2 receptor (PLA2R) antibody levels differed between older (≥50 years) and younger (<50 years) patients. Patients with preformed donor-specific antibodies (DSA) displayed higher chemokine (C-X-C motif) ligand 9 (CXCL9), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and tumor necrosis factor alpha (TNFA) antibody levels than those without preformed DSA (Fig. 1).

There was no information about the individuals based on whom the manufacturer's reference values were established. Therefore, we analyzed the distribution of non-HLA antibody levels in non-transplant patients with ESRD for use as reference values for patients undergoing kidney transplantation. Based on reference values, the healthy subjects displayed 3% to 5% positivity rate for non-HLA antibodies [1, 2]. Most previous studies used manufacturers' reference values [3-5], and non-HLA antibodies are present in 95.2% of hypersensitized patients awaiting kidney transplantation (N=27) [4]. More than one non-HLA antibody has been identified in 13 patients with unexplained positive pre-transplant crossmatch testing results [6]. IFNG, PRKCH, REG3A, and VM antibodies were frequently detected in this study as well as in previous studies [3-5]. All patients had more than one antibody based on the manufacturer's reference values. The positivity rates differed when using the reference values established for patients with ESRD.

CXCL9 and TNFA are related to T-cell reactivity, whereas CXCL9, a proinflammatory chemokine, is produced in response to IFNG. High pre-transplant CXCL9 levels in kidney transplant recipients can predict acute rejection and 5-year graft survival [7]. Tissue injury, alloimmune injury, and other injury types, including those induced by the use of immunosuppressants, after transplantation can cause the exposure of neo-antigens, which can induce the production of non-HLA antibodies [8], thus affecting prognosis [9] and warranting research on *de novo* non-HLA antibodies.

The positivity rates of non-HLA antibodies, including IFNG, PRKCH, REG3A, and VM antibodies, were high in patients with ESRD according to the manufacturer's reference values. We observed differences in certain non-HLA antibody levels according to sex, age, and the presence of DSA. Our findings indicate the need for appropriate reference values for patients with ESRD and revealed the clinical significance of non-HLA antibodies for ESRD patients awaiting transplant by confirming true positivity rates of non-HLA antibodies.

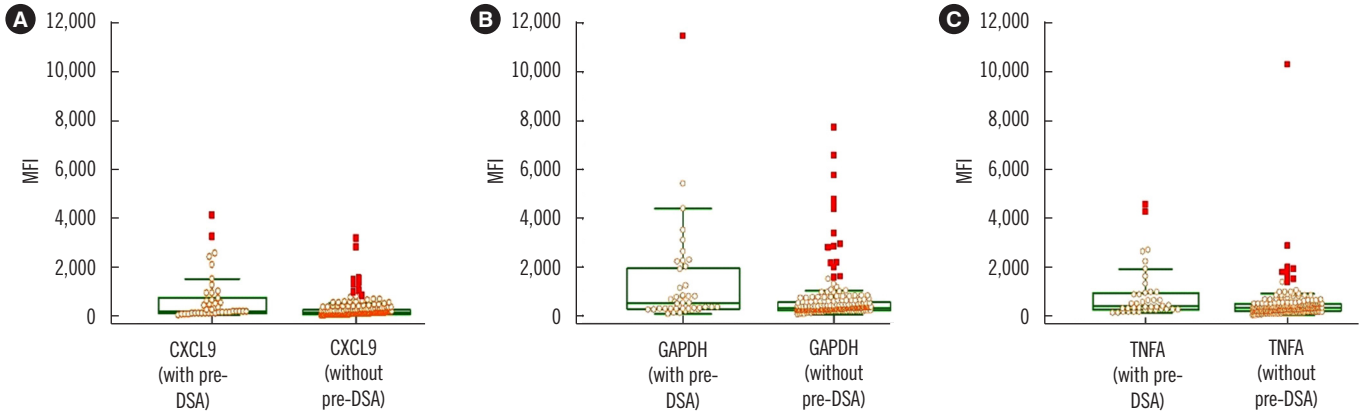

Fig. 1. Non-HLA antibody levels according to the pre-DSA status. (A) CXCL9, (B) GAPDH, and (C) TNFA levels in patients with and without pre-DSA (median [range]: CXCL9, 197.7 [43.0–4102.7] vs. 135.1 [23.1–3168.2]; GAPDH, 504.6 [72.7–11490.2] vs. 311.6 [27.2–7732.8]; TNFA, 457.6 [159–4629.5] vs. 359.3 [54.7–10403.3]).
Abbreviations: CXCL9, C-X-C motif chemokine 9; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MFI, median fluorescence intensity; TNFA, tumor necrosis factor alpha; DSA, donor-specific antibodies.

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AUTHOR CONTRIBUTIONS

Lee HJ and Kim HH designed the study. Kim IY, Choi BH, Kim IY, and Choi BH were involved in data collection. Lee HJ and Shin KH analyzed the data. Lee HJ and Shin KH wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

CONFLICTS OF INTEREST

None declared.

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