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Text:

Oropharyngeal candidiasis (OPC) accounts for about 50% of opportunistic infections among patients with HIV/AIDS, with higher rates in developing countries [1]. Candidiasis is generally a feature of extreme immunodeficiency, with recovery as patients respond to antiretroviral therapy (ART) [2,3], but the problem has not disappeared [4,5]. Few studies have addressed mechanisms underlying this important condition in patients beginning ART under current WHO guidelines. Identification of protective immune responses requires consideration of the fungal burden and the response to ART. Here we focus on salivary IgA, as levels are low in immunodeficient HIV patients with pseudomembraneous candidiasis [6]. IgA can inhibit adherence of microorganisms to buccal epithelium and penetration of the oral mucosa [7]. We explored the role of *C. albicans*-reactive IgA in HIV patients beginning ART in an Asian clinic.

We investigated ART-naïve HIV-infected adults (n=82) recruited consecutively when they began ART at Cipto Mangunkusumo Hospital, Jakarta, Indonesia [8] in January 2013 - January 2014. Inclusion criteria included age >18 years and <200 CD4 T-cells/µl. The cohort had a median(range) age of 31(19-49) years) and 67 (2-199) $CD4^+$ T-cells/µl. 73 individuals were re-examined after 3 months when their counts had increased to 189 (7-601) $CD4^+$ T-cells/µl. We included healthy control subjects (n=40) matched with the patients by age and gender. Controls declared no risk factors for HIV.

OPC was detected by clinical examination, and candida and fungal burdens were determined following culture on CHROMagar and saboroud-dextrose agar (respectively). Individuals were divided according to *C.albicans* burden (<50 or > 50 CFU/ml saliva), in accordance with previous publications [eg: 7]. Specific IgA and IgG in saliva and plasma (respectively) were quantified with in-house ELISAs based on plates coated with a crude *Candida albicans* antigen (Jena Bioscience, Germany). Total IgA was assessed using plates coated with goat anti-human immunoglobulin (Invitrogen, Carlsbad, CA). Plates were blocked with bovine serum albumin and plasma samples were run alongside standard pools of plasma or saliva assigned values of 1000 arbitrary units (AU/ml) for each assay. Bound antibody was detected using horseradish peroxidise-conjugated anti-human IgA (Sigma, St Louis, MI) followed by TMB substrate. Data were analyzed using Prism 5 (GraphPad Software, La Jolla, CA) with non-parametric Mann–Whitney tests for unpaired samples (ie: to compare groups) and Wilcoxon signed rank tests to assess changes on ART in the 73 patients who were followed over time. All data are presented as median (range).

When the groups were assessed without consideration of candidiasis, ART-naïve HIV patients had the

lowest level of total IgA in saliva and the highest level of plasma Candida-reactive IgG, whilst levels of Candida-reactive IgA were similar in all groups (Figure 1A-C). When individuals were divided according to *C.albicans* burden (<50 or > 50 CFU/ml saliva), levels of Candida-reactive IgA were lower in ART-naïve patients with a high *C. albicans* burden (Figure 1E). Similarly levels of Candida-reactive IgA were lower in ART-naïve patients with oral candidiasis than those without [2916(239-16666) *vs* 3378(200-35431) AU/ml, p=0.03]. Whilst these differences were not apparent in controls or after 3 months on ART (Figure 1D, 1F), they are consistent with salivary IgA being protective in untreated patients.

In contrast, HIV patients and controls with a high *C. albicans* burden had higher levels of plasma Candida-reactive IgG than those with a low burden (Figure 1 G-I). Moreover levels of IgG declined on ART in parallel with the incidence of candidiasis (Figure 1C). These findings suggest that plasma Candida-reactive IgG is not protective, but rather reflects the presence of oral candidiasis. Similarly when we divided HIV patients and controls by their total fungal burden, subjects with high burden had higher Candida-reactive IgG than those with a low burden (p=0.02 for healthy controls, p=0.01 for ART naïve and p=0.009 for 3 months on ART; data not shown).

Comparisons with previous studies must consider many factors. Levels of Candida-reactive IgG, IgM or IgA in plasma and saliva were not affected by candidiasis in a study of HIV patients (~ 500 $CD4^+$ T-cells/µl), AIDS patients and healthy controls. However the assays were based on optical density achieved with a single dilution of the sample, so high and low values may be curtailed [9]. IgA responses were not deficient in a cross-sectional study on HIV patients (most on ART) and control subjects with candidiasis [10]. Antibody titers were again expressed as optical densities but were corrected for saliva albumin levels. Correction for total protein also eliminated the small increase in saliva Candida-reactive IgA seen in asymptomatic HIV patients when compared with controls [11] in a study based on a commercial assay that permitted accurate quantitation. Here salivary total protein levels were similar in all groups (data not shown), so we did not adjust the saliva Candida-reactive IgA for salivary total protein levels.

A cross sectional study by Mahajan *et al* [6] also showed low levels of sIgA in HIV patients with and without oral candidiasis, but in our cohort study we observed dynamic changes of saliva Candida-reactive IgA and plasma Candida-reactive IgG in subjects with and without oral candidiasis over time along with the recovery of immune system. Overall, Candida-reactive salivary IgA was lower in untreated HIV patients with OPC and high *C.albicans* burden and recovered 3 months after ART. However Candida-reactive plasma IgG was high in untreated HIV patients along with the high incidence of OPC, and the level decreased on ART. Hence salivary Candida-reactive IgA is potentially protective against OPC.

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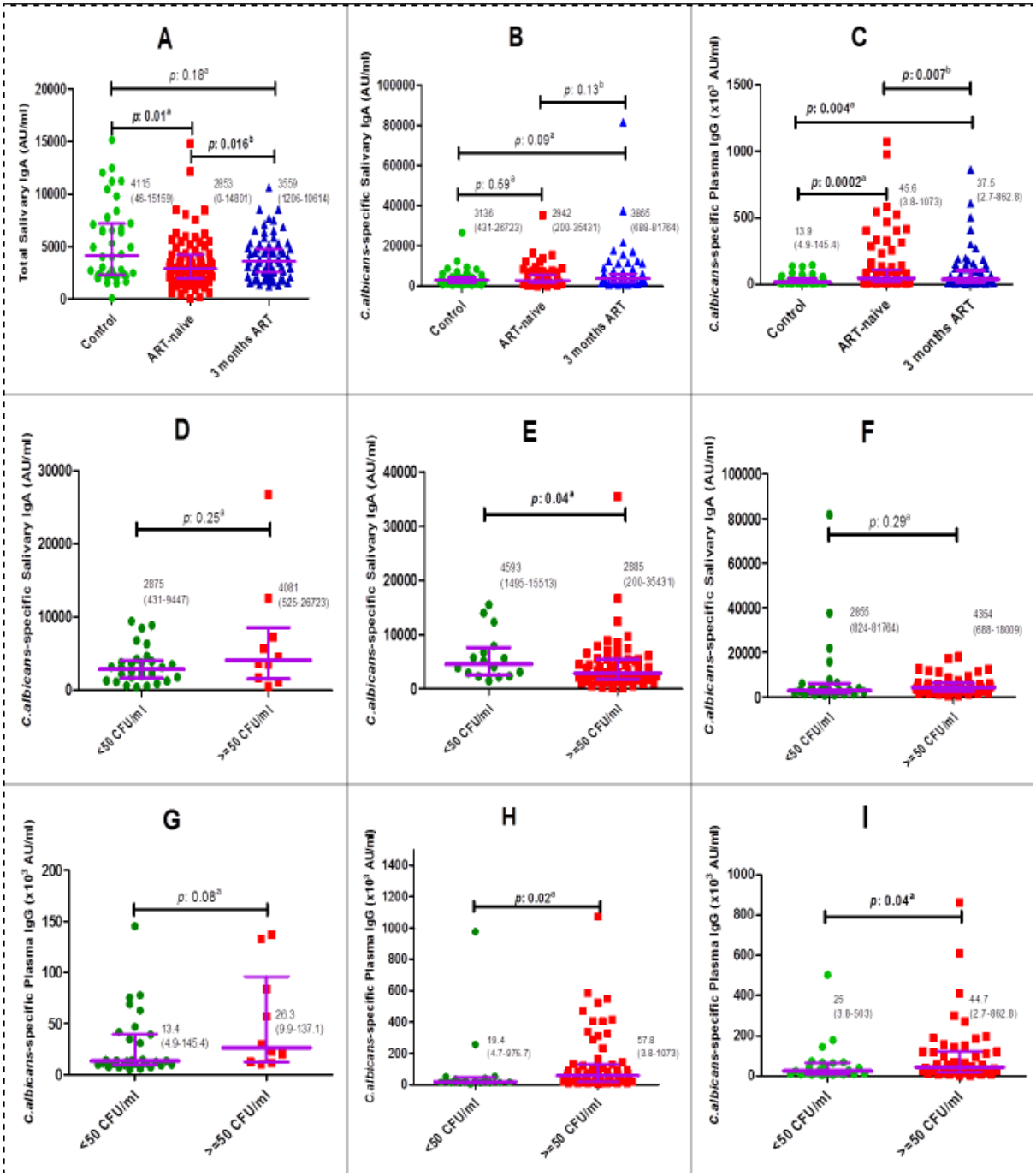
Figure 1: Total IgA (A), Candida-reactive IgA (B) and Candida-reactive IgG levels (C) in healthy controls, ART-naïve patients and patients tested after 3 months on ART. Candida-reactive salivary IgA in the presence of a mild-to-moderate or strong Candida burden in (D) healthy controls, (E) ART-naïve patients and (F) patients tested after 3 months on ART. Candida-reactive plasma IgG in the presence of a mild-to-moderate or strong Candida burden in (G) healthy controls, (H) ART-naïve patients and (I) patients tested after 3 months on ART. [a]Mann-Whitney test, [b]Wilcoxon Signed rank test. Median (range) values are shown in grey.


A
Total Salivary IgA (AU/ml)
p: 0.18a
p: 0.01a
p: 0.016a
4116 (46-15159)
2853 (0-14801)
3559 (1206-10614)
Control
ART-naive
3 months ART
B
C.albicans-specific Salivary IgA (AU/ml)
p: 0.13b
p: 0.09a
p: 0.59a
3136 (431-26723)
2942 (200-35431)
3865 (688-81764)
Control
ART-naive
3 months ART
C
C.albicans-specific Plasma IgG (x10³ AU/ml)
p: 0.007b
p: 0.004a
p: 0.0002a
13.9 (4.9-145.4)
45.6 (3.8-1073)
37.5 (2.7-862.8)
Control
ART-naive
3 months ART
D
C.albicans-specific Salivary IgA (AU/ml)
p: 0.25a
2875 (431-9447)
4081 (525-26723)
<50 CFU/ml
>=50 CFU/ml
E
C.albicans-specific Salivary IgA (AU/ml)
p: 0.04a
4593 (1495-15513)
2885 (200-35431)
<50 CFU/ml
>=50 CFU/ml
F
C.albicans-specific Salivary IgA (AU/ml)
p: 0.29a
2855 (824-81764)
4354 (688-18009)
<50 CFU/ml
>=50 CFU/ml
G
C.albicans-specific Plasma IgG (x10³ AU/ml)
p: 0.08a
13.4 (4.9-145.4)
26.3 (9.9-137.1)
<50 CFU/ml
>=50 CFU/ml
H
C.albicans-specific Plasma IgG (x10³ AU/ml)
p: 0.02a
19.4 (4.7-976.7)
57.8 (3.8-1073)
<50 CFU/ml
>=50 CFU/ml
I
C.albicans-specific Plasma IgG (x10³ AU/ml)
p: 0.04a
25 (3.8-503)
44.7 (2.7-862.8)
<50 CFU/ml
>=50 CFU/ml