Non-Hepatic Reservoir for Hepatitis Delta Virus (HDV) Identified in Human Salivary Gland. Is This Alternative Reservoir a Risk Factor for HBV Co-Infection?

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Introduction

Hepatitis Delta Virus (HDV) is the only known satellite-RNA virus to infect humans and is estimated to affect >60 million people worldwide.[1] This 1700nt virus produces two antigens: the large (L-HDAg) and small (S-HDAg) HDV antigens.[2] A helper virus (ex. Hepatitis B Virus (HBV)) is required for packaging and transmission due to the limited genome size and antigen expression capacity.[2] Recently, HDV has been detected in disease-affected salivary gland tissue of primary Sjogren's disease patients without a detectible current or past HBV co-infection.[3] This is the first demonstration of HDV localization outside of hepatic tissue or blood associated with a disease phenotype.

Primary Sjögren's Disease (SjD) is an autoimmune disease, predominantly affecting women and is associated with decreased tear and/or saliva production, salivary gland localized inflammation, and chronic fatigue. This retrospective study was designed to further characterize HDV antigen and RNA localization and associated clinical features of SjD and viral hepatitis.

Methods

Forty-eight (48) formalin-fixed paraffin-embedded (FFPE) human labial minor salivary gland (LMSG) biopsies from SjD patients were obtained. Immunohistochemical detection of HDV antigens (HDAg), cellular and subcellular markers, and *in situ* hybridization of HDV genomic RNA (RNAscope™) were conducted. Clinical characteristics of the SjD patient cohort were analyzed in comparison to patients' HDV profile. Clinical characteristics examined included: patient demographics, liver biomarkers, and HBV testing information. Patients were stratified by HDAg intensity observed in immunohistochemical staining (HDV Negative, Moderate HDV, High HDV).

Results

Table 2. Patient cohort demographics and clinical characteristics stratified by HDV antigen intensity level.

Demographics	Total (n = 48)	HDV Negative (n = 17)	Moderate HDV (n = 15)	High HDV (n = 16)	*p*
Sex					
Male	4 (8.3%)	1 (5.9%)	1 (6.7%)	2 (12.5%)	ns
Female	44 (91.7%)	16 (94.1%)	14 (93.3%)	14 (87.5%)	
Race					
Asian	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	ns
Black	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Native American	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Pacific Islander	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
White	45 (93.8%)	15 (88.2%)	14 (93.3%)	16 (100.0%)	
Other	3 (6.3%)	2 (11.8%)	1 (6.7%)	0 (0.0%)	
Ethnicity					
Hispanic/ Latino	5 (10.4%)	3 (17.6%)	1 (6.7%)	1 (6.3%)	ns
Not Hispanic/ Latino	43 (89.6%)	14 (82.4%)	14 (93.3%)	15 (93.8%)	
Age (M ± SD)	49.9 ± 13.2	57.2 ± 12.2	42.1 ± 13.3	49.5 ± 10.4	
Age Group					
0 - 19	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.026
20 - 39	10 (20.8%)	0 (0.0%)	6 (40.0%)	4 (25.0%)	
40 - 59	25 (52.1%)	9 (52.9%)	7 (46.7%)	9 (56.3%)	
60+	13 (27.1%)	8 (47.1%)	2 (13.3%)	3 (18.8%)	
Liver Biomarkers					
AST U/L (M, 95% CI)	38.8 (3.8 - 49.3)	35.3 (23.0 - 47.6)	42.5 (15.3 - 69.6)	39.0 (9.3 - 54.6)	ns
Abnormal (%)	5 (12%)	2 (13%)	2 (15%)	1 (7%)	
ALT U/L	40.2 (29.3 - 51.0)	37.7 (22.2 - 53.3)	34.6 (17.1 - 52.1)	48.0 (3.5 - 71.3)	ns
Abnormal	13 (31%)	4 (27%)	3 (23%)	6 (43%)	
ALP U/L	112.2 (50.6 - 130.9)	105.9 (95.2 - 116.6)	102.7 (70.4 - 135.0)	127.9 (39.5 - 174.3)	ns
Abnormal	12 (29%)	3 (20%)	3 (23%)	6 (43%)	
Bilirubin mg/dL	0.7 (0.3 - 0.8)	0.8 (0.5 - 1.0)	0.7 (0.5 - 0.8)	0.7 (0.4 - 0.8)	ns
Abnormal	4 (10%)	2 (13%)	1 (8%)	1 (7%)	
Albumin g/dL	3.9 (3.5 - 4.1)	4.0 (3.8 - 4.2)	3.9 (3.6 - 4.2)	3.9 (3.5 - 4.1)	ns
Abnormal	6 (14%)	2 (13%)	2 (15%)	2 (14%)	
Protein g/dL	6.8 (6.2 - 7.0)	6.8 (6.6 - 7.0)	6.9 (6.4 - 7.4)	6.8 (6.3 - 7.0)	ns
Abnormal	6 (14%)	2 (13%)	2 (15%)	2 (14%)	
HBV Testing					
Tested for HBV	20 (48%)	7 (47%)	7(54%)	6 (43%)	
HBSAg+	0/19	0/6	0/7	0/6	
HBcAb+	0/15	0/5	0/5	0/5	
HBV Diagnosis (ICD-9/10)	0	0	0	0	

Abbreviations: M = mean, SD = Standard Deviation, CI = confidence interval, ALT = alanine aminotransferase, ALP = alkaline phosphatase, AST = aspartate aminotransferase, HBV = hepatitis B virus, HBSAg = hepatitis B surface antigen, HBcAb = hepatitis B core antibody

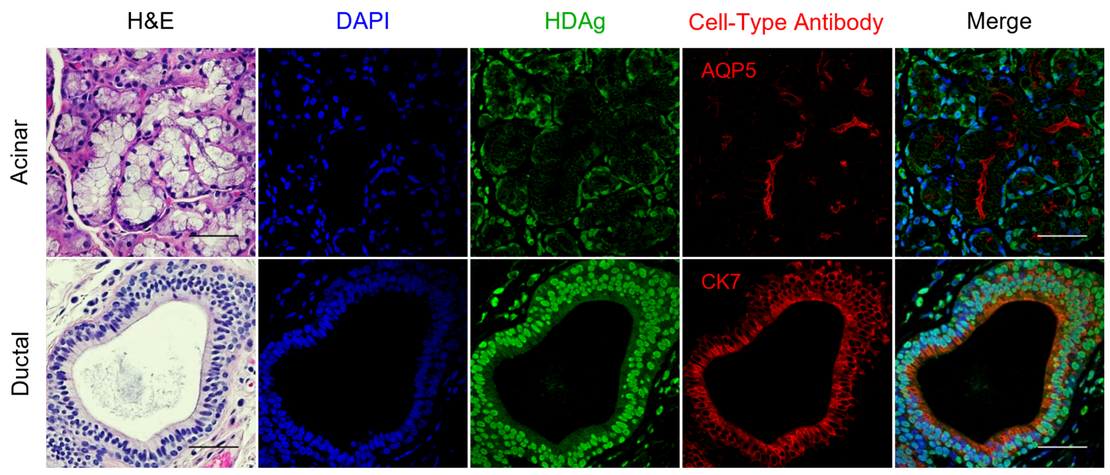

Figure 1. Cellular localization patterns of HDAg in minor salivary gland biopsies of pSS patients. *In situ* H&E and immunohistochemical staining of FFPE minor salivary gland sections. Cell nuclei were stained with DAPI (blue); anti-HDAg antibody was used for immunostaining of HDAg (green). Antibodies for AQP5 and CK7 were used for identification of acinar and ductal cells, respectively (red). Scale bars = 50µm.

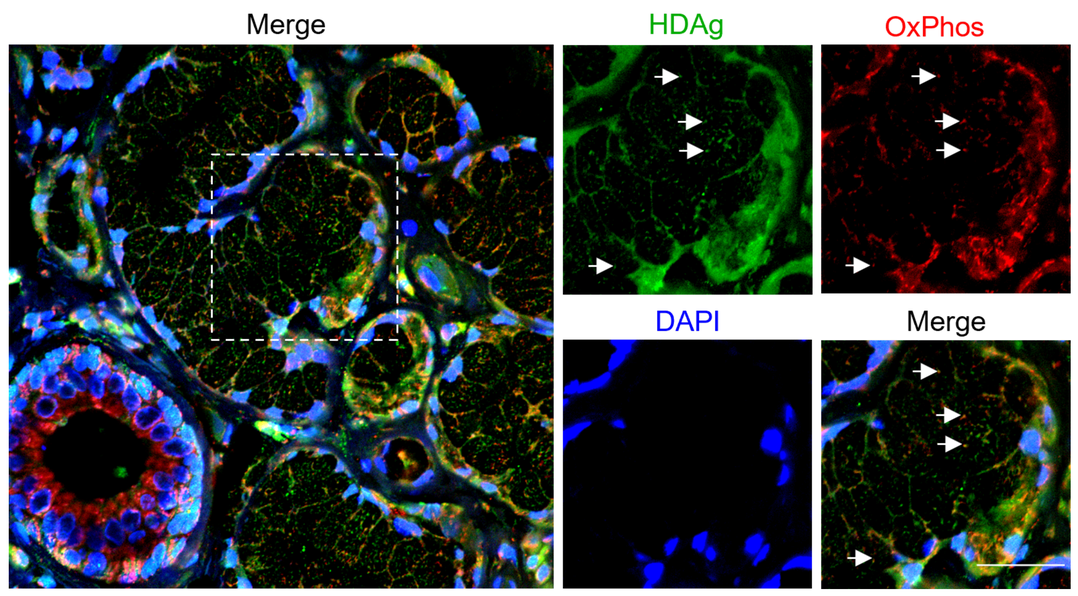

Figure 2. Subcellular colocalization of HDAg with mitochondria. *In situ* immunohistochemical staining of FFPE salivary gland sections. Cell nuclei were stained with DAPI (blue); Anti-HDAg antibody was used for immunostaining of HDAg (green). OxPhos Human Antibody Cocktail was used for identification of mitochondria (red). Arrows indicate examples of colocalized HDAg and mitochondria. Scale bar = 25µm.

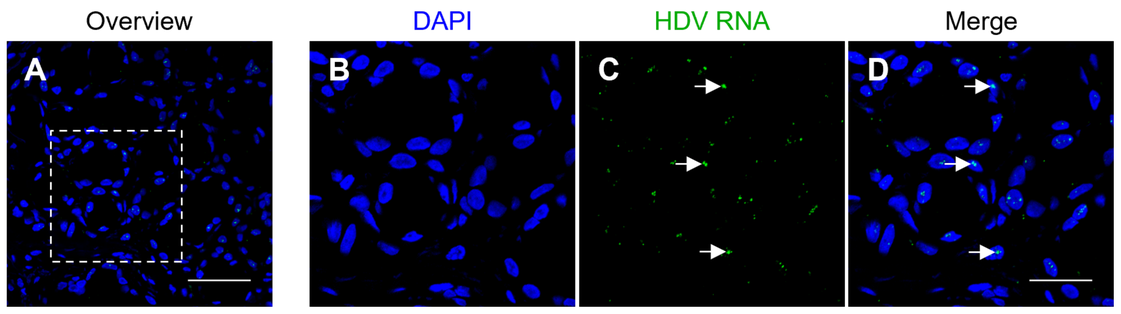

Figure 3. Subcellular colocalization of HDV genomic RNA with nucleus in salivary gland biopsies of pSS patients. A) Merged overview of MSG tissue with cell nuclei (blue) and HDV RNA (green); scale bar = 100µm. Cell nuclei were stained with DAPI (B) and in situ hybridization was conducted with a probe for HDV genomic RNA (C). D) Merged image displaying colocalization patterns of nuclei and HDV RNA. Arrows indicate examples of colocalization of genomic RNA with cell nuclei; scale bar = 50µm

Conclusion

Hepatitis Delta Virus antigen and sequence were detected throughout the SjD LMSG tissue, including in acinar, ductal, and adipose cells (not shown). On a subcellular level, HDAg was observed to localize in the nucleus, cytoplasm, and mitochondria with varying degrees of intensity. HDV genomic RNA sequence was detected and localized to the nuclei. A negative correlation was exhibited between HDAg intensity and patient age. No significant correlations between HDAg and liver enzymes (ALT, ALP, AST, albumin, bilirubin) were identified. HDV + SjD patients tested negative for markers of a current or past HBV co-infection (HBSAg, HBSAg-Ab, HBc-Ab, etc).

References

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