

Mycobacterium avium Paratuberculosis (MAP) and Cytomegalovirus (CMV) Are Frequently Detected in the Saliva of Patients Recently Diagnosed with Crohn Disease (CD) Whereas Oral Propionibacterium Acnes (PA) or Methylacetate (MA) in Their Breath Is Rare

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Abstract

We investigated the presence of MAP, CMV, or Epstein-Barr virus (EBV) in the saliva of patients presenting with periodontal disease, gingivitis or oral aphthosis. We also measured methylacetate in their breath and looked for the presence of PA on their tongue. All patients were prospectively enrolled into a two-year cohort study. We compared the group presenting with CD versus the group without CD. 167 patients were enrolled. 24 patients presented with CD. CMV and MAP were found concomitantly in 67% of CD patients (17 patients) whereas only 1 patient (<0.1%; $p < 0.001$) has these infectious agents in the comparative group. PA was rare in CD (25% versus 70.6%; $p < 0.001$). MA was low, especially with an empty stomach (0.59 ppm +/- 1.45 versus 5.14 ppm +/- 8.97; $p < 0.001$). It was concluded that the association MAP + CMV was frequently found in oral lesions of CD at an early stage. Detection of PA, MAP and CMV, as well as breath test could be easily performed and may participate to the early diagnosis of CD.

Keywords

Mycobacterium avium, Cytomegalovirus, Crohn, Propionibacterium, VOC

1. Introduction

MAP [1]-[11], CMV or EBV [12]-[18] has been reported in CD. Early oral manifestations are frequent in CD [19]-[25], especially in young patients [26]-[35]. The ability of physicians to recognize Crohn's oral lesions is

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poor [30] and the adequate diagnosis could suffer undesirable delays. We investigated whether MAP, CMV or EBV is present in early oral CD. In addition we investigated whether the oral microbiota was altered. PA was selected as a marker of the oral anaerobic flora in CD [36]. The gut microbiota which is altered in CD [37], was investigated by a breath test with the measure of methylacetate, a derivative of methane known to be decreased in CD [38] [39].

2. Methods

All patients consulting a private gastroenterologist and presenting with oral lesions (periodontal disease, gingivitis or oral aphthosis) were prospectively enrolled into a two-year cohort study. No patient had previously received any immunosuppressive agent. Only patients without *Helicobacter pylori* were considered (confirmed by gastro-duodenal biopsies or breath test: Helikit[®]) in order to avoid a bias between CD and not-CD group.

Saliva was collected with swabs. Polymerase Chain Reaction (PCR) was run for MAP, CMV and EBV (Amplix[®] from Alldiag[®]; reagents: Bioneer[®] and Adiavet[®]) in a central laboratory.

The percentages of patients with CD (confirmed by ileoscopy) infected with either MAP, CMV or EBV were compared to the percentages of patients without CD.

PA on the tongue was identified by its specific fluorescence when exposed to black light (Wood lamp; wavelength 320 - 450 nm; peak 365 nm) [40].

Breath test was performed with an ambulatory device (MX6 from Gazdetect[®] France). Methylacetate was measured with an empty stomach and two hours after the intake of lactulose and fructose as previously described [41] [42].

Comparisons of percentages were performed with a Student's t-test and Yates correction.

3. Results

167 patients with oral lesions were enrolled. No patient had previously received any immunosuppressive agents. All patients were *Helicobacter pylori* negative. CD was diagnosed in 24 patients.

MAP alone was found in 4 patients with CD. CMV was found alone once in CD. EBV was never found alone in CD. The combination MAP + EBV + CMV was only found once.

The association MAP + CMV was found in 16 patients with active CD.

Only one patient without CD was found positive with MAP + CMV. This patient presented with isolated palatine aphthosis which abated with acyclovir therapy. Results are detailed in **Table 1**.

PA was less frequently detected in CD (25% versus 70.4%; $p < 0.001$); see **Table 2**.

Patients with CD breathe out less methylacetate, especially with an empty stomach (0.59 ppm +/- 1.45 versus 5.14 ppm +/- 8.97; $p < 0.001$); see **Table 3**.

Table 1. Description of the PCR results according to the diagnosis of CD.

	Neither MAP, CMV or EBV	MAP	EBV	CMV	MAP + EBV	MAP + CMV	MAP + EBV + CMV	CMV + EBV	Total
CD	2	4	0	1	0	16*	1	0	24
Other diseases	111	12	5	6	7	1*	0	1	143
Total	113	16	5	7	7	17	1	1	167

* $P < 0.001$.

Table 2. Detection of oral *Propionibacterium acnes* (PA) according to the diagnosis of CD.

	PA detected	PA not detected	Total
CD	6*	18	24
Other diseases	101*	42	143
Total	107	60	167

* $P < 0.001$.

Table 3. Detection of methylacetate (MA) in breath test according to the diagnosis of CD (measures in ppm: parts per million).

	MA in breath on an empty stomach	MA in breath, 2 hours after lactulose and fructose intake	Hydrogen in breath on an empty stomach	Hydrogen in breath, 2 hours after lactulose and fructose intake
CD	0.59 +/- 1.45*	1.73 +/- 1.92	4.63 +/- 9.99	17.16 +/- 19.24
Other diseases	5.14 +/- 8.97*	4.29 +/- 6.28	4.54 +/- 7.96	18.40 +/- 22.03

* $P < 0.001$.

4. Discussion

Oral lesions, including periodontitis, are frequently associated with CD [19]-[35], CMV or EBV infection [43]-[52].

MAP or CMV has been associated with CD. Whether these infections are opportunistic or may be involved in the development of CD is unknown. These infectious agents are rather considered as environmental modifying agents [6] [7].

This study shows that the association MAP + CMV is frequently found in the saliva of patients with recent active CD, presenting with oral lesions and before any immunosuppressive therapy.

Detection modalities of MAP are still debated [4] [8] [11]. However, since MAP infections could be aggravated by immunosuppressive agents such as anti-TNF-alpha [53], attempts to detect chronic infectious agents should be performed before the use of these medications.

PA is less frequently found in CD. Having a better oral hygiene, patients with CD, may imbalance their oral flora [54]. A decreased prevalence of PA, which belongs to cariogenic flora [55], is compatible with the "hygiene hypothesis" [56] [57] suggested in CD.

CD is associated with a low prevalence of *Helicobacter pylori* infection [58]. All patients infected with *Helicobacter pylori* were therefore excluded to avoid a potential bias when comparing the two groups.

Exhaled MA was very low in CD, especially on an empty stomach. Specific VOC (1-octene, 1-decene, (E)-2-nonene) have excellent accuracy for predicting the presence of Inflammatory Bowel Diseases [37]. Methane is known to be decreased in CD [59].

This study confirms that CD is associated firstly with a high frequency of oral MAP + CMV infection, secondly with decreased oral PA and thirdly with MA decrease in exhaled breath. These detections may be performed with ambulatory inexpensive, non-invasive and easy-to-use devices.

Larger studies are necessary to confirm these preliminary results.

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