GUT FUNCTION & HIV PATHOGENESIS: Nutritional Implications

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September, 2013
GIT & HIV PATHOGENESIS

- **GIT dysfunction** has been recognized as a major manifestation of HIV infection, since the earliest recognition of the acquired immune-deficiency syndrome (AIDS).

- It was originally thought, that this disease manifestation was considered to be sequelae of the immune destruction, that characterizes AIDS, **rather than it being central to the pathogenesis of AIDS**. 1
In the past decade, it has been observed that the mucosal immune system and the intestinal immune system are pivotal in the pathogenesis of AIDS, with the most critical events, namely:

- Transmission,
- Viral amplification,
- CD4+ T-cell destruction occurring in the gastrointestinal tract (GIT),
- Breakdown of the mucosal barrier with consequent microbial translocation,

are considered to be major drivers of AIDS progression.¹

The mucosal tissue is not only a primary site of viral transmission, but also a major site of viral replication, and CD4+ T-cell destruction, regardless of the route of transmission.¹
HIV Enteropathy

- GIT enteropathy in persons living with HIV (PLWH) can occur from the acute phase of infection, through to advanced disease.

- It is characterized by:
  - diarrhoea,
  - increased GIT inflammation,
  - increased intestinal permeability (up to fivefold higher than in healthy individuals),
  - malabsorption of bile acid, and vitamin $B_{12}$. \(^2\)
HIV Enteropathy

- **Histologically**, the GIT enteropathy in HIV involves:
  - inflammatory infiltrates of lymphocytes
  - damage to the GIT epithelial layer (which includes villous atrophy, crypt hyperplasia and villous blunting).

- These **pathological changes may** occur in the absence of any detectable bacterial, viral or fungal enteropathogens, which are often associated with enteropathy.\(^2\)
HIV Enteropathy

- Although the mechanisms that cause the abnormalities in HIV enteropathy, are poorly understood, it has been suggested that HIV has a “virotoxic” effect on the enterocytes.
- HIV gp120 has been found to result in increased concentrations of calcium in the enterocytes, which is associated with tubulin depolymerisation, and a decrease in epithelial cells’ ability to maintain ionic balances. ²
HIV Enteropathy

- Local activation of GIT immune system, is also thought to play a role in HIV enteropathy.
- In HIV there are high levels of proinflammatory mediators such as beta chemokines interleukin-6 (IL-6), interleukin-10 (IL10) and interferon (IFN-\(\gamma\)) found in the lamina propria of the colon in PLWH.
- The degree of inflammation has been found to correlate with the level of viral replication.
- Although systemic immune activation is a hallmark of HIV, the etiology of the latter remains elusive. ²
HIV Enteropathy

- It has also been postulated that local bacterial translocation across the damaged tight epithelial barrier, results in microbial products that stimulate the immune system locally, presumably through receptors such as Toll-like receptors.
- A crucial consequence of induction of local inflammation through any means, is through HIV’s preferential infection of activated CD4+ T-cells, which in turn augments the HIV replication.\(^2\)
CD4+ T-cell Destruction

- Originally it was thought that HIV involved a period of latency, however, it is now well established that HIV attaches to the CD4+ molecule on the T-cells and the monocyte and macrophage lineage cells, and on a chemokine receptor, during acute infection.
- The direct infection of CD4+ T-cells leads to the destruction of these cells and global immune deficiency, as these cells are required for induction and control of most immune responses.
- The infection of the monocyte and macrophage lineage cells appears to be particularly important in chronic HIV infection, and are possibly major reservoirs for viral replication and persistence, and hence contributing to immune deficiency. 1
CD4+ T-cell Destruction

- More recently, it has been observed that the CD4+ T-cells which bear the CCR5 HIV co-receptor, are the primary targets of HIV.
- The CD4+ T-cells with CCR5 receptors constitute the majority of the CD4+ T-cells. It is estimated that nearly 80% of the T-cell population, are found in the GIT. 3
- Depletion of the CD4+ T-cells involves the entire GIT. 2
- Significant depletion of these cells occurs in the first 17 days post HIV infection, and in a recently postulated model (based on GIT biopsies), it is thought that the bulk of CD4+ T-cell depletion occurs in the first 2 to 3 weeks of acute infection. 3
CD4+ T-cell Destruction

- PLWH with a **CD 4 count of less than 200 cells/uL**, have been found to have a **twofold increase in diarrhoea**.
- The latter affirms the view that diarrhoea is an AIDS defining condition.
- A **decrease in CD4+ T-cells** (less than 200 cells/uL) has been observed to be associated with **intestinal parasite infection** (for example: *Cryptosporidium, I belli & S. Stercoralis*), and with a higher incidence of diarrhoea.\(^4\)
Th17 Cell Loss and Impairment of Mucosal Integrity

- HIV mediated loss of Th17 cells from the gut-associated lymphoid tissue (GALT) has been observed to impair mucosal integrity, and innate defense mechanisms against gut microbes.
- Th17 cells are important for intestinal homestasis.
- Th17 cells are involved in epithelial regeneration, and stimulate the production of defensins and mucin, as well as induce the expression of claudins, which are components of epithelial tight junctions.
- The Th17 cytokine, interleukin-22 (IL-22) increases the production of the lipopolysaccharide binding protein (LBP) in the liver.  

[^5]
Th17 Cell Loss and Impairment of Mucosal Integrity

- Considering, the massive CD4+ T-cell depletion in the lamina propria after HIV infection; it is probable that Th17 cells are also depleted by HIV.
- Th17 cells have multiple roles in controlling epithelial integrity and microbial invasion, the depletion of Th17 is likely to affect the integrity of the GIT.
- To date, Salmonella has been directly shown to translocate across the GIT barrier, when Th17 cell function is compromised in the GALT, in PLWH. 5
Structural Villus Changes

- In PLWH with AIDS, the mean villus/height and mean villus/crypt ratios have been found to be significantly lower than those in non-HIV infected (normal) controls. 6
Figure 1.

- Biopsy of small bowel from a patient with AIDS and pathogen-negative diarrhoea.
- Note the prominent villus atrophy, crypt architectural distortion, decrease in crypt/villus ratio, and the significant influx of lymphocytes within the lamina propria.

Impact of HIV Infection on Lactose Absorptive Capacity

- It has been reported that lactose malabsorption is significantly higher (70%) in PLWH, than HIV-uninfected controls (34%).
- Furthermore, the degree of lactose malabsorption was found to be significantly greater in PLWH with advanced disease, versus those in the earlier stages of disease.
- The degree of lactose malabsorption has been found to be related to whether PLWH were symptomatic and had intestinal manifestations, than asymptomatic PLWH and non-HIV infected controls.
- It is presumed that apart from the presence of the HIV, other factors (probably both structural and immune), determine the enterokinetic alterations responsible for lactase deficiency and lactose malabsorption.\(^7\)
Clinical Presentation of Enteropathy

- A high percentage of PLWH worldwide have been reported to initially present or will develop diarrhoea, irrespective of whether they are on HAART or not.
- In the United States, 50% of PLWH have presented with diarrhoea.
- However, in developing countries a prevalence of as many as 80% of PLWH have presented with diarrhoea.
- The presentation of diarrhoea may or may not be in the presence of an opportunistic infection of the GIT. 6
Clinical Presentation of Enteropathy

- **Clinical presentations** vary among PLWH with HIV-associated diarrhoea, depending on the principal section of the GIT involved.

- **Small bowel diarrhoea** tends to result in:
  - large bulky postprandial stools almost immediately after eating,
  - individual may experience postprandial paraumbilical *abdominal pain*.
  - if the affected individual fasts, the diarrhoea significantly decreases.
  - Individuals with small bowel diarrhoea usually experience *weight loss*. ⁶
Clinical Presentation of Enteropathy

- Individuals with large intestine diarrhoea (termed “colitic diarrhoea”), usually present with:
  - frequent, small-volume stools, and the stools may have visible blood and mucus.
  - these individuals will usually experience lower-quadrant abdominal pain, and the sensation of rectal urgency.
- In many instances, it may be difficult to differentiate between small- and large bowel diarrhoea.  

\[6\]
Clinical Presentation of Enteropathy

- The opportunistic infections that affect the GIT in PLWH include:
  - **parasitic infections** - for example: Cryptosporidia, Isopora and Cyclospora,
  - **viral** - in particular Cytomegalovirus \{CMV\},
  - **bacterial** - for example: Mycobacterium tuberculosis \{TB\}, Salmonella, Shigella, Campylobacter jejuni and Mycobacterium avium complex \{MAC\}.  

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6. [footnote]
Clinical Presentation of Enteropathy

- Prompt treatment of CMV is very important, as it is associated with a poor prognosis in PLWH and a high rate of recurrence.
- PLWH with GIT MAC usually have disseminated disease, a very low CD4⁺ T-cell count, and limited survival time.
- Since the introduction of HAART, the incidence and prevalence of MAC has decreased.
- TB of the GIT may affect immune-compromised and immune-competent individuals.
Clinical Presentation of Enteropathy

- Opportunistic infections are not limited to the small- and large-bowel.
- May occur in the **upper GIT**, including:
  - **oesophageal pathology** – e.g.: *candida oesphagitis*, CMV, and herpes simplex virus,
  - **Gastric and duodenal pathology** e.g.: CMV, *Helicobacter pylori* and *cryptosporidium*.
- These infections may lead to **dysphagia** and hence **poor nutritional intake** but also **recurrent dehydrating vomiting**.
Clinical Presentation of Enteropathy

- Most of the GIT opportunistic infections will result in further aggravation of HIV-associated enteropathy, due to structural damage and/or immune sensitization;
- Hence making it difficult to differentiate whether the severity of GIT related symptoms, is due to HIV disease progression or the severity of other opportunistic infections.
Accelerated GIT Transit Time

- In a recent study (2009), it was reported that HIV-infected children with a higher severity of malnutrition and more advanced stages of HIV clinical symptoms, had **accelerated whole gastrointestinal transit time**.

- Early nutritional intervention for children with severe malnutrition and advanced HIV disease, with specialized lactose-free feeds with a low osmolality, to aid in delaying gastric GIT transit time, and to allow for greater nutrient absorption, was recommended. ⁹
The Role of Gut in HIV Disease Progression

- It has been speculated that due to the massive depletion of memory T-cells in the gut, as well as structural defects of the GIT lining, microbial translocation from the gut is probably involved in driving immune activation.

- It is thought that the **gut-derived microbes or microbial products translocate** to the systemic circulation in the absence of overt bacteremia.
Figure 2: Change in cell integrity, and bacterial translocation, in acute & chronic infection.
Quantification of Microbial Translocation

- Microbial translocation can be quantified by measuring **plasma levels** of **lipopolysaccharide (LPS)**, the endotoxin produced by bacteria that have translocated across the GIT lining.

- In PLWH with acute HIV infection, were found to have LPS levels similar to those of non-HIV infected individuals, however, in PLWH with **chronic HIV infection**, the LPS levels were **significantly higher**. ³
Quantification of Microbial Translocation

- In an earlier study, in which non-infected HIV individuals were injected with LPS, with a resultant plasma level of LPS as low as **14pg/ml**, that produced systemic immune activation with increased levels of inflammatory cytokines (for example, tumour necrosis factor, interleukin (IL)-1 receptor antagonist, IL6 & IL8).

- In studies on **PLWH with chronic infection**, the **LPS levels** were found to be **75pg/ml**; hence sufficient to stimulate systemic immune activation. ³
Quantification of Microbial Translocation

- It is well known that immune activation decreases with potent antiviral therapy (ART), although the decline is much slower than HIV RNA levels, and may remain elevated for a year after ART.
- It has been observed that statistically significant decreases in LPS levels only occur in PLWH, after 48 weeks on ART.
- It appears that ART is currently the most effective way to protect the gut, and help reduce bacterial translocation, and hence reduce chronic systemic immune activation.  

3
HAART and the Gastrointestinal Tract

- Currently ART has in most cases, been found to reduce plasma viral loads to undetectable levels, resulting in subsequent increases in peripheral blood CD4+ T-cells.
- Early studies of HIV-associated enteropathy after the initiation of ART, showed significant decreases in GIT symptoms, namely, abdominal bloating and cramping, and loose stools.
- However, a decrease in viral replication and CD4+ T-cell reconstitution, does not occur at a similar rate at all anatomic sites, especially in the GIT.²
HAART and the Gastrointestinal Tract

- Recent studies have shown that in the small bowel CD4+ T-cell reconstitution was poor.
- PLWH with acute HIV infection who had been on highly active antiviral therapy (HAART), had a much greater reconstitution (twofold) of CD4+ T-cells compared to individuals with chronic HIV infection.
- Importantly, it was also observed that although many PLWH treated with HAART reconstituted peripheral CD4+ T-cells, but no HIV-infected individual ever reconstituted GIT CD4+ T-cells to levels observed in non-infected individuals.2
HAART and the Gastrointestinal Tract

- **GIT CD4$^+$ T-cells** have been observed to still produce HIV virus, even years after the initiation of HAART.

- Although the GIT is well vascularised, and ART drugs should be bioavailable; **high levels of multidrug-resistant proteins**, also named, “toxin pumps” (such as P-glycoprotein), are expressed on the apical surface of columnar epithelial cells of both the small and large intestine.

- It is speculated that these multidrug-resistant proteins, have specificity for protease inhibitors and nucleoside analogs, and may reduce the local concentration of ART drugs to infected cells in the GIT.

- Hence, allowing the **virus to slowly replicate and limit reconstitution of CD4$^+$ T-cells**. ²
HAART and the Gastrointestinal Tract

- Recently findings, also suggest that the **fibrotic deposition of collagen** also occurs in the **GIT Peyer’s patches**, even during the acute phase of HIV infection.
- The degree of architect damage of the Peyer’s patches, **predicts** **GIT CD4+ T-cell depletion after HAART**.
- Although **HAART reduces GIT immune activation**, it is thought that the **ability of remaining (but damaged) lymphoid to support significant CD4+ T-cell reconstitution**, is **permanently damaged**. ²
- As many as **30% of PLWH on HAART**, fail to **reconstitute CD4+ T-cells**, despite **HIV-viremia control**, and are described as **immunologic-nonresponders (INRs)**. **INRs have an increased risk of HIV/ AIDS progression**. ¹¹
Plasma Citrulline: A Biomarker of Enterocyte Mass in PLWH?

- Plasma or serum citrulline essays have recently emerged as the best tool in assessing enterocyte mass, irrespective of the etiology of the intestinal mucosal disease.

- Citrulline is the metabolic product of glutamine, and its related amino acids, and arginine, and is specifically synthesized by small bowel enterocytes.

- Citrulline has been validated for quantitative enterocyte assessment in villous atrophy disease. 12
Plasma Citrulline: A Biomarker of Enterocyte Mass in PLWH?

- Citrulline remains uninfluenced by nutritional status,
- level of hypoalbuminemia,
- or inflammatory status.

- The only limitation is significant renal failure (creatinine clearance of < 30ml/min), because citrulline is metabolized into arginine in the proximal convoluted tubules in the kidneys. ¹²
In a recent study (2009) it was found that plasma citrulline essays were a **reliable indicator of severe chronic infectious enteropathy in PLWH**, and hence a **reliable predictor** for the **indication for parenteral nutrition (PN)** for such cases.

- A **low citrulline level** of <10umol/L is considered an **indication for PN**.

- Whilst for an individual with a citrulline level of >10umol/L, **enteral route nutrition** supplementation is recommended. *Normal citrulline levels = 40umol/L*.

- Citrulline is easy to measure through ion-exchange or reverse-phase liquid chromatography, which can usually be **performed in most hospital biochemistry laboratories**.  

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12
The Role of HIV Vaccines in Enhancing GIT Mucosal Cell Immunity

- There is some promising evidence that suggests that direct surgical introduction of a vaccine (replication-competent recombinant adenovirus \( \text{rAd} \) vectors, specifically rAd5), rather than oral gavage, results in 100-fold higher transgene expression, and which stimulates potent CD8\(^+\) T-cell responses in the intestinal and systemic compartments.

- These responses could be further enhanced through intramuscular rAd5 injections.\(^{13}\)

- The activation status of CD8\(^+\) T-cells is considered to be one of the best predictors of HIV disease progression.\(^{5}\)
New Therapies: Gene modification

- The safety & tolerability of infusions of lentiviral vector modified autologous CD4 T cells (VRX496-T) in HIV pts with controlled viremia were demonstrated.
  - Gene modified cells were shown to exert genetic pressure on HIV-1.

New Therapies: Bovine immunoglobulin

- Oral serum bovine immunoglobulin improves duodenal immune reconstitution & absorption function in RVD pts with HIV enteropathy
  - 2.5g x 2 times daily for 8wks
  - SBI significantly increased intestinal mucosal CD4 lymphocyte counts, function & showed evidence of intestinal repair in pts with HIV enteropathy.

Role of Probiotics in Gut Integrity & Immune function...in HIV

- Modulate gut microflora by inhibiting proinflammatory cytokines,
- Reduce gut permeability,
- Reduction in abdominal bloating & discomfort, and diarrhoea,
- Antibacterial property/ resistance to pathogenic bacteria,
- Essential vitamin synthesis (Wilson NL et al, J Assoc Nurs in AIDS Care, 2013).
Role of Probiotics in Gut Integrity & Immune function...in HIV

- Probiotics may aid in significantly reducing/preventing oral candidiasis,
- Use of probiotics as an adjunct therapy in HIV maybe:
  - Low cost
  - Non-invasive
  - Effective intervention for treating HIV related Sx that impact on the quality of life.

(Wilson NL et al, J Assoc Nurs in AIDS Care, 2013).
Probiotics in HIV - Evidence

  - DBRC trial pilot,
  - *Lactobacillus rhamnosus* & *Bifodobacterium lactis* & prebiotic (10g inulin),
  - 4 groups: probiotics, prebiotics, symbiotic (pre & probiotic) & placebo, Rx = 16wks
  - Subjects: ARV naïve, CD4 >350
  - Findings: Probiotic group:
    - Significant increment of probiotic load
    - Significant decrease in harmful bacteria,
    - Significant decrease in interleukin 6 cytokine
Probiotics in HIV - Evidence

- Irvine SL et al, Nutr Research 2011
  - Probiotic yoghurt consumption – impact on GI Sx, productivity & nutritional intake PLWHA – Mwanza, Tanzania
  - Lactobacillus Ramnosus
  - Subjects; 85 Rx grp, 86 Control (placebo) grp, 70% in both grp on ARV, average CD4 370
  - Consumed yoghurt (200ml) x 4/ wk
  - Yoghurt made & supplied by established community kitchen
Probiotics in HIV - Evidence

- Irvine SL et al, Nutr Research 2011

- **Outcomes** – Probiotic grp:
  - Median increase 2hrs/ day – capacity to work
  - Decrease in febrile Sx by 1 dy
  - Subgrp on ARVs = *less like to experience severe stomach/ abdominal pain* vs control grp.

- **Potential benefits**
  - Community kitchen model = economically feasible, effective means for an aspect of HIV intervention
  - Yoghurt = *well tolerated*, also = improved nutritional intake & may help alleviate GI Sx related to HIV & ARVs
Probiotics in HIV - Evidence

- Hemsworth JC et al, Gut Microbes, 2012 (prelim data – 1st phase)
  - Micronutrient supplemented probiotic yoghurt
  - RDBC trial – 3 period (Ontario Canada),
    - Probiotic grp,
    - Vitamin (Vit A,C) grp,
    - Probiotic + Vitamin grp
  for 30dys with a 14 day washout
- Subjects: n=21, *varying CD4* (<200 to >500), on ARVs, *average wt* = 70kg (well nourished).
Probiotics in HIV - Evidence

- Hemsworth JC et al, Gut Microbes, 2012 (pre-lim data – 1st phase)
  - **Outcome (1st Phase):**
    - Overall increase in CD4
    - Average wt gain = 0.7kg
    - All grps:
      - subjective *increase in energy levels & ability to perform tasks*,
      - yoghurt was well tolerated.
Probiotics in HIV - Evidence


- Probiotics appear to support the maintenance of strong epithelia layer & stimulate innate immunity, which acts as the 1st layer of defense against translocation of viral particles & bacterial pathogens.

- L rhamnosus GR-1 & Bifidobacterium bifidum confer some immunostimulatory activity in children & adults

- L rhamnosus GG
  - most effective in reducing duration of diarrhoea
  - Stimulates immunoglobulin Ig A production
Probiotics in HIV – Need for Caution

- Patients may develop bacteremia on Probiotics (e.g. case report of a 29yr old female on Lactobacillus rhamnosus).
  (Wilson NL et al, J Assoc Nurs in AIDS Care, 2013).
- There is limited evidence re use of probiotics in HIV
- Further research is required to establish the efficacy & safety in HIV
- Establish ideal strain & dose & mode (capsule vs enriched yoghurt), stable in bile & able to withstand pH of stomach & small intestines, sufficient adherence to intestinal mucosa.
Probiotics in HIV – Need for Caution
Imai K et al, Cell Mol Life Sci 2012 (Japan)

- Observed that high butyric acid producing bacteria may be involved in AIDS progression by reactivating the latent HIV provirus.
- Invitro study
- Observed high butyric acid producing bacteria (Fusobacterium nucleatum, Closteridium cochlearium, Eubacterium multiforme {gut}, and Anaerococcus tetradius {vagina}) could promote gene expression of latent HIV-1, thus making co-infection of these anaerobic bacteria one of the risk factors for promoting AIDS progression.
Summary

- **Hand and food hygiene** are v. important.
- Consider *yoghurt/ maas* or low lactose therapeutic feeds in under weight PLWHA.
- A *low lactose therapeutic feed with MCT fat* will be better tolerated in a severely emaciated PLWHA (avoid high fat supplements).
- Consider *monitoring citrulline levels* in severely emaciated PLWHA.
References


5) Hofer U & Speck RF. Disturbance of the gut-associated lymphoid tissue is associated with disease progression in chronic HIV infection. Semin Immunopathol 2009; 31:257-266.


References


References


Thank you for your attention!