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Short Communication

Serum siderocalin levels in patients with tuberculosis and HIV infection

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ABSTRACT

Objectives: *Mycobacterium tuberculosis* produces high-affinity siderophores that play essential roles in iron acquisition and tuberculosis (TB) pathogenesis. In response, host cells secrete a siderophore-binding protein, siderocalin, to limit the bacteria's access to iron. The objective of the present study was to evaluate the levels of siderocalin in patients with TB with or without HIV infection compared to controls.
Methods: Siderocalin levels were tested using a neutrophil gelatinase-associated lipocalin (NGAL) ELISA kit in four populations: HIV-infected patients with TB (HIV^{pos}, TB^{pos}), non-HIV-infected patients with TB (HIV^{neg}, TB^{pos}), HIV-infected patients without TB (HIV^{pos}, TB^{neg}), and healthy controls (HIV^{neg}, TB^{neg}).
Results: Serum siderocalin levels were significantly elevated in patients with TB regardless of their HIV status (HIV^{neg}, TB^{pos} 920 (480–1050) pg/ml; HIV^{pos}, TB^{pos} 494 (166–1050) pg/ml), whereas lower levels of siderocalin were seen in HIV-positive patients (HIV^{pos}, TB^{neg} 268 (77–937) pg/ml; HIV^{neg}, TB^{neg} 453 (193–994) pg/ml).
Conclusions: The results indicate that active TB leads to an up-regulation of serum siderocalin regardless of HIV status, whereas HIV infection leads to a down-regulation of serum siderocalin levels in both TB-negative and TB-positive individuals. Further studies are needed to evaluate siderocalin as a potential marker of active TB and to clarify its role in the pathogenesis of HIV-associated TB.

Introduction

Tuberculosis (TB) continues to be a significant cause of morbidity and mortality worldwide. The recent annual report on TB by the World Health Organization (WHO) estimated 10 million new and recurrent TB cases globally, with India alone accounting for 27% of these cases, and 1.3 million and 300 000 deaths in HIV-negative and HIV-positive individuals, respectively (WHO, 2018). The progression from uninfected or latently infected to active TB is often insidious and has minimal symptoms in the initial months, resulting in delayed diagnosis and poor outcomes (Lui et al., 2014). Thus, the identification of markers involved in the pathogenesis and disease progression of TB could be very valuable in the early diagnosis and treatment of the disease.

Iron acquisition by *Mycobacterium tuberculosis* plays an important role in TB pathogenesis. Carboxymycobactin is a salicylate-type siderophore that is secreted by the bacteria in order to facilitate the capture and uptake of iron from the environment (Boelaert et al., 2007). *M. tuberculosis* strains that are unable to produce or utilize carboxymycobactin have been found to grow poorly under iron-restricted conditions and to exhibit attenuated growth under both in vitro and in vivo conditions in mouse models of TB (De Voss et al., 2000), confirming the role of siderophore-mediated iron acquisition in *M. tuberculosis* virulence and growth. On the other hand, mammals have evolved mechanisms to limit the availability of iron to pathogens such as *M. tuberculosis*. These mechanisms include lowering of serum iron concentrations and increased secretion of the protein siderocalin (also known as lipocalin 2 and neutrophil gelatinase-associated

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lipocalin, NGAL) into extracellular fluids (Golonka et al., 2019; Wilson et al., 2016).

Siderocalin, which is expressed by a number of cell types, including macrophages and neutrophils, has the ability to bind to bacterial siderophores such as carboxymycobactin, thus preventing them from delivering iron to pathogens (Holmes et al., 2005). It has been shown to inhibit the intracellular growth of mycobacteria residing in macrophages and alveolar epithelial cells (Saiga et al., 2008; Johnson et al., 2010). Moreover, siderocalin-deficient mice have increased susceptibility to infection with *M. tuberculosis* (Saiga et al., 2008). In humans, circulating neutrophil counts and siderocalin levels have been inversely correlated with the risk of infection among contacts of TB patients (Martineau et al., 2007).

HIV infection is well known to be associated with enhanced susceptibility to TB, but it is not clear whether alterations in siderocalin levels contribute to this susceptibility. Also, there is evidence to show that serum siderocalin concentrations are significantly reduced in HIV-infected individuals and improve following antiretroviral therapy (Landro et al., 2008).

To further examine this issue, the serum siderocalin levels were measured in patients with TB, HIV, TB and HIV co-infection, and healthy controls.

Methods

Serum siderocalin levels were quantified in four sets of individuals: HIV-infected patients with TB (HIV^{pos}, TB^{pos}), non-HIV-infected patients with TB (HIV^{neg}, TB^{pos}), HIV-infected patients without TB (HIV^{pos}, TB^{neg}), and healthy controls (HIV^{neg}, TB^{neg}). The study was conducted at the Christian Medical College, Vellore and was approved by the Institutional Review Board and Ethics Committee. Informed consent was obtained from all subjects.

Serum samples from recruited patients were stored at −70 °C and were used at the end of the study period for estimations. TB was diagnosed by mycobacterial culture or Xpert MTB/RIF assay. Siderocalin was assayed using the NGAL ELISA kit (KIT 036; BioPorto Diagnostics, Denmark) according to the manufacturer's instructions. The lower and upper limits of detection were 10 pg/ml and 1000 pg/ml, respectively. Values outside these limits were recorded as being out of range.

Results

A total of 159 adults with a mean age of 40 years were included in the study and were broadly divided into HIV-negative and HIV-positive groups for analytical purposes; the groups were further subdivided according to their TB status (TB or control). Among the HIV-negative subjects, there were 39 TB-positive cases and 40 healthy controls; in the HIV-positive group, 37 were TB-positive cases and 43 were without active TB.

In the absence of TB, the median siderocalin concentration in healthy HIV-negative controls and HIV-positive individuals was 453 (193–994) pg/ml and 268 (77–937) pg/ml, respectively ($p < 0.001$) (Figure 1a). TB infection considerably increased the siderocalin concentrations in each group. Specifically, HIV^{neg}, TB^{pos} patients had a median siderocalin concentration of 920 (482–1050) pg/ml, which was significantly higher compared to the HIV^{neg}, TB^{neg} controls ($p < 0.001$). Similarly, HIV^{pos}, TB^{pos} patients had a median siderocalin concentration of 494 (166–1050) pg/ml, much higher than the HIV^{pos}, TB^{neg} controls ($p < 0.001$) (Figure 1b). In addition, the difference between the mean siderocalin concentrations of HIV^{neg}, TB^{pos} (920 (480–1050) pg/ml) and HIV^{pos}, TB^{pos} (494 (166–1050) pg/ml) patients was found to be statistically significant ($p < 0.001$).

The median CD4 count in the HIV^{pos}, TB^{neg} controls was 302 (26–451) cells/μl, and among HIV^{pos}, TB^{pos} subjects was 93 (13–445) cells/μl. There was no difference in the proportions of pulmonary and extrapulmonary TB cases between HIV-negative and HIV-positive patients, with 45% pulmonary TB and 65% extrapulmonary TB in each group. The difference in neutrophil counts between the HIV^{neg}, TB^{neg} and HIV^{neg}, TB^{pos} groups was also found to be statistically significant (Table 1).

Discussion

In this study, the highest levels of siderocalin were observed in TB patients without an underlying HIV infection, followed by TB patients with an HIV infection, and the lowest level was found in HIV patients without TB. This finding confirms previous observations that HIV infection decreases circulating siderocalin levels (Landro et al., 2008) and also suggests a possible contribution of the HIV infection-induced decline in siderocalin to increased susceptibility to TB. In a similar study, Halaas et al. (2010) also found that siderocalin inhibits the growth of *Mycobacterium avium* in both in vitro and in vivo conditions, but is ineffective in inhibiting the intracellular mycobacteria, as the mycobacteria escape siderocalin by choosing a different pathway.

There was a robust and significant increase in circulating siderocalin levels in TB patients with or without HIV (Figure 1a, Table 1), suggesting that HIV infection does not completely prevent the up-regulation of siderocalin production that presumably contributes to an increase in its circulating levels. However, HIV infection does seem to compromise the up-regulation of siderocalin, as indicated by the lower levels of siderocalin in the HIV^{pos}, TB^{pos} population compared to that of the HIV^{neg}, TB^{pos} population

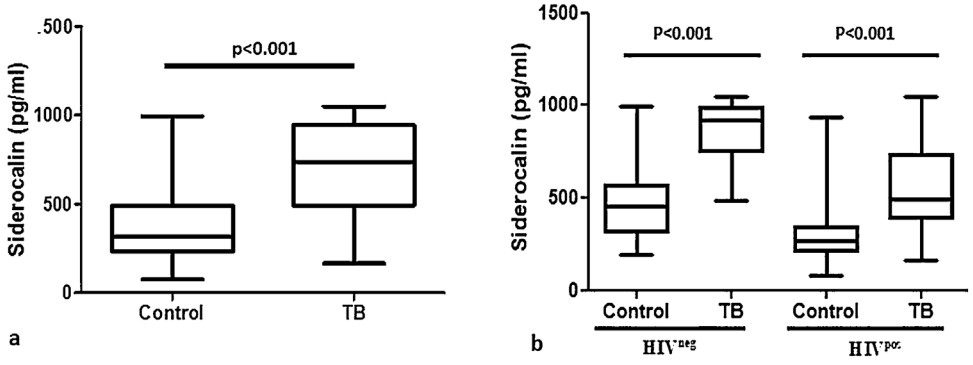

Figure 1. Serum siderocalin concentrations among cases (active TB) and controls. (a) Overall cases and controls with or without HIV infection. (b) Cases and controls among HIV-negative and HIV-positive subjects separately.

Table 1
Patient characteristics.

	HIV-negative (*n* (%) or median (range))			HIV-positive (*n* (%) or median (range))		
	Control *n* = 40	TB *n* = 39	*p*-Value	Control *n* = 43	TB *n* = 37	*p*-Value
Age (years), mean ± SD	39.4 ± 13.5	43.0 ± 16.5	0.375	39.1 ± 7.4	37.9 ± 7.2	0.341
Sex						
Male	23 (58%)	27 (69%)	0.352	35 (81%)	30 (81%)	1.000
Female	17 (42%)	12 (31%)		8 (18%)	7 (19%)	
Smoking	3 (13%)	6 (23%)	0.472	1 (3%)	6 (20%)	0.042
Diabetes mellitus	2 (9%)	10 (26%)	0.182	5 (12%)	2 (5%)	0.442
ANC (cells/μl)	3894 (2286–6286)	7004 (3150–22 532)	<0.001	3025 (1300–7931)	3896 (988–12 052)	0.013
CD4 count (cells/μl)	–	–	–	302 (26–451)	93 (13–445)	<0.001
Serum siderocalin (pg/ml)	453 (193–994)	920 (482–1050)	<0.001	268 (77–937)	494 (166–1050)	<0.001

TB, tuberculosis; SD, standard deviation; ANC, absolute neutrophil count.

(Figure 1b, Table 1). This abnormality may contribute to poor control of *M. tuberculosis* replication. The HIV-associated mechanisms that impair TB-induced up-regulation of siderocalin, as well as the potential prognostic significance of siderocalin levels in relation to susceptibility to TB in HIV-infected individuals, are issues that are worth further investigation.

Conclusion

In conclusion, the trend in siderocalin levels suggests that there is an up-regulation of the protein in individuals with active TB, with or without HIV infection. In HIV infection, there is an inhibition of siderocalin expression, and hence the level of siderocalin in patients with TB and HIV co-infection is lower than the level in those with active TB without HIV, indicating an independent effect of HIV infection on siderocalin. The mechanism and possible prognostic significance of this variation needs further study. More studies in this field could lead to siderocalin being used as a prospective potential marker of active TB, and its possible usage as an adjunctive therapy to standard chemotherapy in TB.

Author contributions

George M. Varghese: Study conceptualization, designing, execution, supervision of data collection, reviewing the data analysis, and manuscript writing. Vijay Prakash Turaka, Jeshina Janardhanan, and Vijayakumar T. S.: Data collection and review of manuscript. Sadhana Yadav: Reviewing the data analysis and manuscript writing. Kavitha M. Lakshmi: Data analysis. Bobby Cherayil: Study conceptualization, reviewing the data analysis, and reviewing the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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