Serum lipocalin-2 levels are decreased in patients with leprosy

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Abstract

Background: Leprosy is an infectious disease caused by *Mycobacterium leprae* affecting the skin, peripheral nerves and mucosae. Lipocalin-2 is a key component of the immune system's antimicrobial defence - it prevents iron uptake by binding and sequestering iron-scavenging siderophores and thus inhibits bacterial growth.

Aim: We evaluated serum lipocalin-2 levels in leprosy patients and its relationship to the pathogenesis and prognosis of the disease.

Materials and methods: In this case-control study, serum lipocalin-2 levels were measured by ELISA in 20 patients with leprosy and 20 healthy controls.

Results: Serum levels of lipocalin-2 were significantly reduced ($P < 0.001$) in leprosy patients as compared to controls. The levels were significantly higher ($P < 0.014$) in patients with multibacillary leprosy than in those with paucibacillary leprosy. Although the levels of lipocalin-2 were higher in patients with multiple nerve involvement as compared to those with involvement of 1 or 2 nerves, the results were not statistically significant.

Limitation of the study: The small sample size and the lack of different ethnic groups in the study were the major limitations of this study.

Conclusion: The lower lipocalin-2 concentrations in leprosy patients point to the importance of the protective functions of lipocalin-2. The elevated levels of lipocalin-2 observed in leprosy patients with neural involvement may be related to the reported neurodegenerative role of lipocalin-2.

Key words: Serum, lipocalin-2, leprosy, neutrophil gelatinase-associated lipocalin

Plain Language Summary

Mycobacterium leprae causes leprosy, which is an infectious illness affecting the skin, peripheral nerves and mucosa. Lipocalin-2 is a key component of the immune system's antimicrobial defence. It inhibits bacterial growth through the prevention of bacterial iron uptake by binding and sequestering iron-scavenging siderophores. Thus we aimed to evaluate serum lipocalin-2 level in leprosy patients and its relation to disease pathogenesis and prognosis in Egypt. To achieve this aim, this case-control study was carried out comprising 40 individuals: 20 diagnosed with leprosy and 20 healthy controls. Serum lipocalin-2 was measured by ELISA. Results revealed a significant reduction in the concentration of serum levels of lipocalin-2 in cases in comparison with controls. It was significantly higher in multibacillary than in paucibacillary patients. Moreover, it was higher in patients affected with multiple nerve involvement than those with involvement of one or two nerves, but the difference was statistically insignificant. We concluded that the lower lipocalin-2 concentrations in leprosy patients might indicate the protective functions of lipocalin-2. Elevated levels of lipocalin-2 with neural involvement were observed that, though statistically insignificant, might be in line with the reported neurodegenerative role of lipocalin-2.

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Introduction

Leprosy is a chronic infection caused by *Mycobacterium leprae* that involves the skin, peripheral nerves and mucosae of the upper respiratory tract and eyes. It often leads to severe deformities and may result in social stigmatization.[1] Although the total number of cases of leprosy declined globally between 2011 and 2019 period compared to 1987, the prevalence is still high in many countries, including India and Brazil.[2]

Lipocalin-2 is an acute-phase protein generated by inflammation.[3] It has been linked to cell proliferation, differentiation, tissue involution and death and high levels have also been linked to cancer.[4,5] Lipocalin-2 limits the growth and spread of bacteria that rely on siderophore-mediated iron uptake by creating a ternary structure with bacterial siderophore, thus inhibiting iron uptake by bacteria.[6] Lipocalin-2 knock-out mice have been shown to be more susceptible to infection by siderophore-producing bacteria such as *Klebsiella pneumonia*, *Escherichia coli* and *Mycobacterium tuberculosis*.[7–11]

Pathogenic mycobacteria produce two types of siderophores: mycobactin and carboxymycobactin. The former is an envelope-restricted siderophore while the latter is water-soluble. Both sequester and bind soluble as well as protein-bound iron with high affinity and they can each be bound by lipocalin-2.[12,13]

The role of lipocalin-2 in mycobacterial infection models has been studied *in vivo*.[14,15] In cultivated macrophage cell lines, lipocalin-2 inhibits the *in vitro* growth and intracellular replication of *Mycobacterium tuberculosis*. It also limits the growth of *Mycobacterium bovis* BCG in cultured murine alveolar epithelial cells by binding to soluble siderophores.[16]

Siderophores are also present in *Mycobacterium leprae*,[14] but the role of lipocalin-2 in leprosy has not been evaluated. We aimed, therefore, to evaluate serum lipocalin-2 levels in patients with leprosy and study the role of lipocalin-2 in its pathogenesis and prognosis.

Methods

This case-control study was conducted in 20 leprosy patients and 20 healthy age and gender-matched controls attending the Outpatient Clinic of the Kafr El Sheikh Dermatology and Leprosy Hospital, Menoufia, Egypt. The control group also included healthy volunteers. Patients with chronic diseases known to alter the expression of lipocalin-2 such as psoriasis, cholestasis, haematologic disorders, cardiomyopathy, malignancy and chronic kidney disease[17] were excluded.

The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Menoufia University Hospital. All participants gave written informed consent for participation in this study.

A detailed history was taken in all cases that included the name, age, sex, marital status, history of blood transfusion, dental therapy, intravenous drug abuse and previous admission to the hospital for a fever. A complete general and dermatological examination was performed and the bacteriologic index was assessed by slit skin smear.[14]

Serum human lipocalin-2 estimation

Serum human lipocalin-2 was measured by ELISA. Venous blood samples (5 mL) were collected in a plain tube and stored for 30 minutes at room temperature to clot. The sample was centrifuged for 10 minutes at 3000 RPM and the serum was separated and stored at –80°C until analysis. The serum human lipocalin-2 measurement was carried out using commercial ELISA kits (Chongqing Biospes Co., Shanghai, China).

Statistical analysis

Data were collected, and tabulated and statistical analyses were performed using IBM SPSS Statistics version 23 for Windows version 11 (IBM Corp., Armonk, N.Y., USA). The chi-square test (χ^2) was utilized for studying the association between two qualitative variables. Whenever any of the expected cells were less than five the Fischer's Exact test was used. The paired *t*-test was used for comparing quantitative variables of normal distribution within the same group (i.e., before and after injection) for paired quantitative data, while the Student's *t*-test was used for comparing quantitative variables of normal distribution for unpaired quantitative data.

Continuous variables were presented as mean and standard deviation, while categorical variables were presented as frequency (%).

Results

The 20 leprosy cases included 9 males and 11 females with a mean age of 42.55 ± 15.02 years (range 19–61 years). The control group included 5 males and 15 females with a mean age of 37.40 ± 9.10 years (range 29–59 years). There was no significant difference between cases and controls regarding age and sex [Table 1].

The type of lesions seen in the patients is depicted in Table 2. Ten (50%) patients had paucibacillary leprosy and the remaining had the multibacillary disease. Nerve involvement was present in 10 patients (one patient with four affected nerves, four patients with involvement of two nerves and five patients with involvement of three nerves). A single patient had epistaxis.

Positive slit skin smears were seen in 10 patients (three patients with a score of 1+, two each with scores of 2+, 3+ and 4+ and a single patient with a 5+ score).

Serum lipocalin-2 levels

The serum lipocalin-2 levels in patients with leprosy were significantly lower than in controls (488.9 ± 315.1 vs 917.2 ± 592.4 ng/mL; *P*-value = 0.04). The levels were much

Table 1: Comparison between the two studied groups according to age and gender

	Leprosy patients (No. = 20)		Control (No. = 20)		Test of sig.	*P*
	No.	%	No.	%		
Gender						
Male	9	45.0	5	25.0	$\chi^2 = 1.758$	0.185
Female	11	55.0	15	75.0		
Age (years)						
Min.–Max.	19.0–64.0		29.0–59.0		t = 1.311	0.199
Mean ± SD	42.55 ± 15.02		37.40 ± 9.10			
Median	43.50 (28.50–57.50)		33.50 (31.0–42.50)			

No.: number; %: percent; χ^2: Chi-square test; SD: standard deviation; t: Student's *t*-test

Table 2: Clinical data

Clinical features	Number of patients (%)
Type of lesions	
Hypopigmented macules with decreased sensation	9 (40%)
Macules and nodules	4 (20%)
Nodules and infiltration	2 (10%)
Nodules and ulcerations	2 (10%)
Erythematous patch with decreased sensation	1 (5%)
No lesions	1 (5%)
Nerve involvement	
No nerves involved	10 (50%)
2 nerves	4 (20%)
3 nerves	5 (25%)
4 nerves	1 (5%)
Epistaxis	1 (5%)
Type of leprosy	
PB	10 (50%)
MB	10 (50%)

higher in multibacillary as compared to paucibacillary patients (665.3 ± 325.5 ng/mL vs 314.4 ± 188 ng/mL; *P*-value = 0.014*) [Table 3] and did not correlate with the clinical manifestations of leprosy (type of skin lesions, nerve involvement or epistaxis) [Table 4], the age of the patient or the slit skin smear results (*P*-value = 0.14 and 0.133, respectively) [Table 5].

Discussion

Lipocalin-2 is an antimicrobial protein that plays a key role in the innate immune response to bacterial infection.[18] It has been implicated in the progression of infectious diseases and chronic inflammatory pain disorders. The role of lipocalin-2 in bacterial infections, including those caused by *Escherichia coli,*[19] *Mycobacterium bovis* and *Mycobacterium tuberculosis,*[20] has been elucidated, but its role in leprosy has not been investigated earlier. This study was conducted with the aim of estimating the serum levels of lipocalin-2 in patients with leprosy in order to clarify its role in disease pathogenesis and prognosis.

We found that serum lipocalin-2 levels in patients with leprosy (both multibacillary paucibacillary) were significantly lower than in controls. Earlier studies have shown that lipocalin-2 deficient mice were more susceptible to acute *Mycobacterium bovis*[20] and *Mycobacterium tuberculosis* infections.[14] Wu *et al.* have demonstrated that lipocalin-2 enhances the intracellular clearance of *Escherichia coli* by macrophages when added to culture media and that this is mediated by limiting iron availability to the pathogens.[21] This mechanism may be relevant not only for *Escherichia coli* but also for other macrophage infecting pathogens such as salmonella, treponema, chlamydia and mycobacteria.[9,10]

The antibacterial effects of lipocalin-2 may also be attributed to both its effect on neutrophils and its antioxidant properties. It induces the migration of neutrophils to the site of infection by inducing of chemoattractant release from macrophages, epithelial cells and keratinocytes.[22] It also protects against hydrogen peroxide toxicity and induces upregulation of many antioxidant enzymes such as superoxide, dismutase and heme oxygenase.[23,24]

Lipocalin–2 levels were found to be significantly higher in multibacillary leprosy than in paucibacillary leprosy in our study. It was also higher (although not statistically significant, possibly owing to the small sample size) in patients with multiple (>2) affected nerves as compared to those with 1 or 2 affected nerves.

The increased levels of lipocalin-2 in multibacillary leprosy may be related to the extensive nerve involvement seen in this type of leprosy. Lipocalin-2 expression has been shown to increase in spinal neurons following peripheral nerve injury and contributes to the development of pain in experimental mice.[25] It has also been found to be markedly increased in the neutrophils of damaged sciatic nerves, suggesting that lipocalin-2 may also be involved in the early inflammatory response in the peripheral tissues after nerve injury.[26]

Limitations of the study

The small sample size and the lack of different ethnic groups were limitations of this study.

Table 3: Comparison between cases and controls regarding serum lipocalin-2 level (*n* = 40)

Serum lipocalin-2 level (ng/mL)	Leprotic patients			Control (*n* = 20)		*P*
	Total (*n* = 20)	PB (*n* = 10)	MB (*n* = 10)			
Min.–Max.	179.9–1200	179.9–760	180.3–1200	120–1920	U	P1
Mean ± SD	489.88 ± 315.14	314.42 ± 187.96	665.33 ± 325.46	917.18 ± 592.39	116.5*	0.023*
Median (IQR)	299.95 (233.25–763.3)	266.6 (190.3–300)	733.30 (299.9–833.3)	1010 (390–1400)	H	P2
					8.514*	0.014*

n: sample size; SD: standard deviation; H: Kruskal–Wallis test; U: Mann–Whitney *U* test; *P*: *P* value for comparing between the studied groups; *: Statistically significant at $P \leq 0.05$; P1: *P* value for comparing between leprosy patients and controls; P2: *P* value for comparing between PB and MB patients

Table 4: Relation between serum lipocalin-2 level and clinical data of leprosy patients

Lesion	N	Serum lipocalin-2 level (ng/mL)			H	*P*
		Min.–Max.	Mean ± SD	Median		
No	1		299.90		4.660	0.198
Macules nodules	4	266.60–833.30	661.63 ± 264.92	773.30		
Nodules infiltration	2	180.30–960.0	570.15 ± 551.33	570.15		
Nodules ulcer	2	666.60–700.0	683.30 ± 23.62	683.30		
Patches ulcers	1		1200.0			
Erythematous patch with weak sensation	1		199.90			
Hypopigmented macule with weak sensation	9	179.90–760.0	327.14 ± 194.74	266.60		
Nerve involvement						
Less than 3 nerves	14	177.0–1200	419.87 ± 265.3	333.3	U	0.772
More than 3 nerves	6	266.6–888.0	436.96 ± 240.9	316.6	0.290	
Epistaxis						
No	19	179.90–1200.0	499.87 ± 320.50	300.0	–	–
Yes	1		299.90			

N: sample size; U: Mann–Whitney *U* test; SD: standard deviation

Table 5: Correlation between serum lipocalin-2 level and age and slit skin smear among leprotic patients

	Serum lipocalin-2 level (ng/mL)	
	r_s	*P*
Age (years)	0.339	0.143
Slit skin smear	0.348	0.133

r_s: Spearman coefficient

Conclusion

The lower lipocalin-2 concentrations in leprosy patients might indicate the importance of the protective functions of lipocalin-2.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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