Current Topics in Peptide & Protein Research
Vol. 24, 2023

Original Article

Comparative assessment of complement C1q subcomponent subunit B (C1QB) protein in serum exosomes of patients with HIV-tuberculosis coinfection, pulmonary tuberculosis, HIV mono-infection and healthy humans

Shweta Kushwaha[1,2], Rajbala Yadav[1], Roopendra Kumar[1], Anjana Goel[2], Santosh Kumar[3], Devendra Singh Chauhan[1] and Ajay Vir Singh[1,*]

[1]Department of Microbiology and Molecular Biology, ICMR-National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Tajganj, Agra-282004, Uttar Pradesh, India;
[2]Department of Biotechnology, GLA University, Mathura-281406, Uttar Pradesh, India;
[3]Department of TB and Chest, Sarojini Naidu Medical College, Agra-282002, Uttar Pradesh, India.

ABSTRACT

Complement C1q subcomponent subunit B (C1QB) is a crucial component of compliment C1Q which plays an important part in host defense system. The compliment C1Q components have been identified as biomarkers for the diagnosis of active tuberculosis. However, limited information is available about the expression of C1Q components in patients with HIV-TB coinfection and disease controls. The present study was aimed to determine the abundance of C1QB in serum exosomes of patients with HIV-TB coinfection, pulmonary TB, HIV mono-infection, and healthy humans. A total of twenty serum-derived exosome samples from patients with HIV-TB coinfection (n=5), pulmonary TB (n=5), HIV mono-infection (n=5), and healthy humans (n=5) were processed for the detection of C1QB using western blotting method. The beta-actin protein was used as an internal control to normalize the expression of C1QB between different samples of the study groups. The mean densitometric intensity values for C1QB of the study groups were compared using Mann-Whitney test and p-value <0.05 was considered as statistically significant. The results demonstrate that C1QB was present in serum exosomes of all study participants. The mean densitometric band intensity for C1QB was statistically significantly higher in patients with HIV-TB coinfection (p-value: 0.003) and pulmonary TB (p-value: 0.015) as compared to healthy controls. However, the C1QB mean densitometric band intensity in exosome samples of patients with HIV-TB coinfection and pulmonary TB was found to be higher but the difference was not significant (p-value: >0.05) when compared to samples of HIV mono-infected patients. In conclusion, the present study reports increased expression of C1QB in serum exosomes of patients with HIV-TB coinfection and pulmonary TB as compared to HIV mono-infected patients and healthy humans and indicates that C1QB could be a potential adjunct biomarker to discriminate patients with HIV-TB coinfection and pulmonary TB from healthy humans. Further studies are required to establish the diagnostic potential and clinical relevance of altered C1QB expression in serum exosomes of patients with and without HIV-TB coinfection.

KEYWORDS: complement C1q subcomponent subunit B protein, serum exosomes, tuberculosis, HIV-TB coinfection, western blotting.

*Corresponding author: avsjalma@gmail.com; ajayvir.s@icmr.gov.in

1. INTRODUCTION

Despite being curable, tuberculosis (TB) claimed the deaths of 1.6 million people (including 187 000 people with HIV) in 2021 and continues to be a significant public health concern worldwide [1]. The coinfection of human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis* (principal causing agent of TB) accelerated the global incidence and TB-derived mortality and further exacerbated the situation especially in high HIV/TB burden regions (i.e. African and Asian countries) of the world. Early diagnosis paired with appropriate treatment is crucial for effective management of TB in people living with HIV (PLWH). The available diagnostic tests (acid-fast staining, culture, and assay based on proliferation of nucleic acids, Interferon-gamma release assay, QuantiFERON-TB, T-SPOT-TB, etc) have significant shortcomings and offer a suboptimal diagnosis of TB in HIV-positive patients [2-4]. This situation significantly hampered the effective management of TB in HIV-positive individuals and necessitates the discovery of novel biomarkers for the development of newer diagnostics for early and accurate diagnosis of TB in PLWH.

In recent years, numerous studies have described the role of complement system in pathogenesis of several infectious diseases [5-7], and evidences suggested that its components can be a potential biomarker to discriminate active TB patients from latent TB infection and/or healthy humans [8-10]. Recently, several studies have reported significantly higher expression of complement C1q subcomponent subunit B (C1QB) protein (25 kDa polypeptide chain complement C1q) in active TB disease conditions and suggested that C1QB could be a useful biomarker for the diagnosis of active TB [9-11]. However, the status of C1QB expression in cases with HIV-associated TB has been largely unexplored. The present study was aimed to evaluate the level of C1QB protein in serum exosomes of patients with HIV-TB coinfection, pulmonary TB, HIV mono-infection, and healthy humans.

2. MATERIALS AND METHODS

Ethical approval for this research study was obtained from the Institutional Human Ethics Committee, National JALMA Institute for Leprosy and Other Mycobacterial Diseases (NJIL&OMD), Agra, Uttar Pradesh-282001, India (Registration No ECR/257/Inst/UP/2013). Written informed consent was obtained from all the study participants.

2.1. Study population and clinical samples

A total of 20 individuals (15 confirmed patients with HIV-TB coinfection (n=5), pulmonary TB (n=5), HIV mono-infection (n=5), and five healthy individuals) were prospectively enrolled in this study. The study participants were adults (<18 years of age) who had visited Sarojini Naidu Medical College, Agra for diagnosis and/or treatment of TB/HIV. After appropriate counselling and written informed consent, the demographic information of the study participants (Table 1) was recorded. About 3.0 ml of blood sample was collected from each of the study participants and processed for the isolation of serum using centrifuge (3000 g for 15 min) method. The serum samples were processed for the isolation of exosomes using a commercial kit (Exoquick reagent kit, System Biosciences, Mountain View, CA, USA). The exosomes were characterized as per the method described by Kushwaha *et al*. [12], and lysed using radioimmunoprecipitation assay buffer as described by Wu *et al*. [13]. The protein concentration of the exosome lysate samples was estimated using standard Bradford assay (Bio-Rad Laboratories, USA) as per the method of Bradford [14], and the absorbance was measured at 560 nm [14].

2.2. Detection of C1QB protein in serum exosome lysate samples using western blotting method

About 20 µg of protein of exosome lysate was mixed with 2 x Laemmli buffer and heated at 95 ºC for 5 minutes, followed by chilling on ice for 5 min before loading onto a 12% SDS-polyacrylamide gel at constant 90 V at room temperature for 1.5 hr. Subsequently, samples run in SDS-PAGE were blotted onto PVDF membranes (BioRad Laboratories, Pvt. Ltd, India). After blotting, the membrane was blocked in 5% BSA (Hi-Media, India) for 2 hrs. After incubation, the membrane was incubated with C1QB-specific primary antibodies (Proteintech, 1:2000) for overnight at 4 ºC. After that, the

Table 1. Demographic characteristics of the study participants and mean densitometric band intensity for C1QB of different study groups.

Variables	HIV-TB co-infected patients (n=5)	Pulmonary TB patients (n=5)	HIV mono-infected patients (n=5)	Healthy controls (n=5)	Total (n=20)	Mean densitometric band intensity for C1QB	P value
Gender: Male/female	3/2	2/3	3/2	3/2	11/9	1.767/1.688	0.147
The median age in years (male/female)	34/44	48/35	31/43.5	37/29	36/35	-	-
Age: up to 35 and above 36	3/2	2/3	2/3	3/2	10/10	1.825/1.638	0.426
Smoker/ non-smoker	1/4	0/5	0/5	2/3	3/17	2.323/1.675	0.307
Alcoholic/ non-alcoholic	1/4	0/5	1/4	3/2	5/15	1.706/1.740	0.400
With cough/ without cough	1/4	0/5	2/3	0/5	3/17	1.863/1.722	0.344
With weight loss/without weight loss	2/3	5/0	2/3	0/5	9/11	1.725/1.634	0.385

membrane was washed three times with 1x TBST and incubated with goat anti-rabbit IgG HRP-conjugated secondary antibody (1:20000) for 2 hrs at room temperature. After incubation, the membrane was washed three times. After washing, the membrane was incubated with chemiluminescent substrate (BioRad Laboratories, Pvt. Ltd, India). The immunoreactive blots were visualized using the chemiluminescent reagent ECL-Plus (Thermo-Fischer, France). Bands were analyzed using Image Lab Software (Bio-Rad Laboratories). The β-actin was used to normalize the expression of C1QB in samples from different study groups. The experiment was conducted in triplicate and mean values were used for further analysis.

2.3. Statistical analysis

The mean densitometric band intensity values for C1QB protein were used to compare between study groups using Mann–Whitney U unpaired test. Statistical differences were analyzed using GraphPad Prism 9.0 software (GraphPad Software, Inc., La Jolla, CA, USA), and p-value < 0.05 was considered as statistically significant.

3. RESULTS

3.1. Demographic and clinical characteristics of the study participants

The details of demographic and clinical characteristics of study participants are presented in Table 1. Out of the 20 study participants, 11 (55.0%) and 9 (45.0%) were male and female, respectively. The median age of the male participants was 36.0± 13.65 years and that of the female participants was 35±11.95 years. Group-wise, the median age of the male and female patients with HIV-TB coinfection, pulmonary TB, HIV mono-infected, and healthy controls was 34, 48, 31, 37 and 44, 35, 43.5, 29 years, respectively. Majority of the study participants were non-smokers (n=17, 85.0%), non-alcoholic (n=15, 75.0%), and without history (more than two weeks) of cough (n=17, 85.0%), and without history of weight loss (n=11, 55.0%). The abundance of C1QB protein in serum

exosomes was not found to be significantly correlated with any of the different demographic/ clinical characteristics of the study participants (Table 1).

3.2. Detection of C1QB proteins in serum exosomes of the study groups

The altered expression of C1QB protein was found in serum exosomes derived from different study groups (Table 2, Figures 1 & 2). The mean densitometric band intensity for C1QB was statistically significantly higher in patients with HIV-TB coinfection as compared to healthy controls (p-value: 0.003). However, the mean densitometric band intensity for C1QB for samples of HIV-TB co-infected patients was higher, but the difference was not significant when compared to that for samples of HIV mono-infected (p-value: 0.07, t-test) and pulmonary TB (p-value: 0.15) patients (Table 2). The mean densitometric intensities of the bands for C1QB for pulmonary TB patients were significantly higher when compared to healthy individuals (p-value: 0.015). However, no significant difference was found in

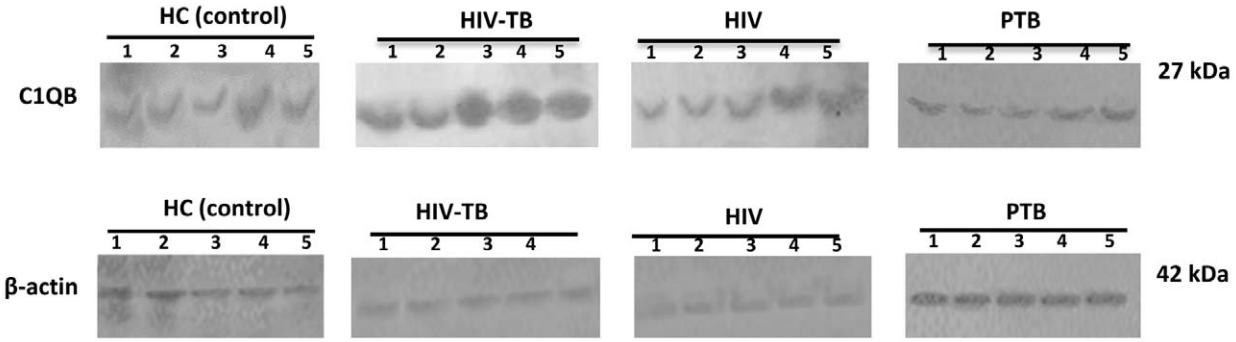

Figure 1. Abundance of C1QB protein in sera-exosomes of healthy control (HC) and HIV-TB co-infected (HIV-TB), HIV mono-infected (HIV) and pulmonary TB (PTB) patients evaluated using western blotting method.

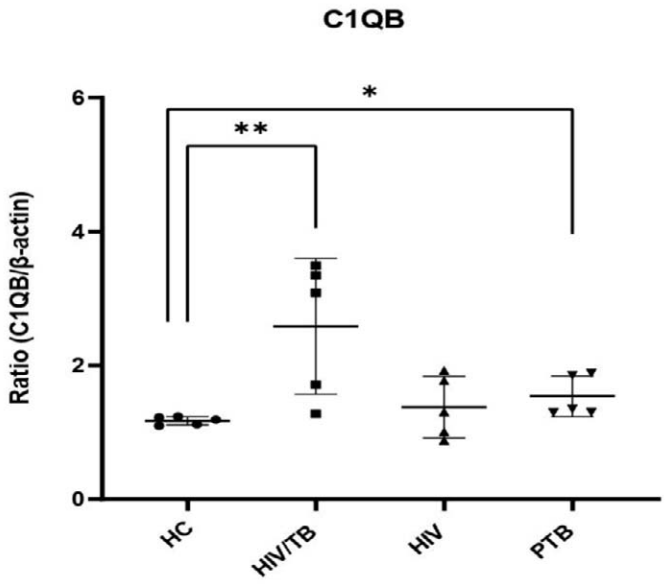

Figure 2. Relative mean densitometric band intensity (C1QB/β-actin) of CIQB protein in serum exosomes of different study groups. Data are plotted as mean ± SD. Statistical significance was determined using the Man-Whitney U test ($P < 0.05$).

Table 2. Comparative evaluation of mean densitometric band intensity for C1QB in different study groups.

Study groups	Mean densitometric intensity for C1QB	Fold change	*p*-value
HIV-TB coinfection vs Healthy controls	2.583382/1.174544	2.19	0.003
Pulmonary TB vs Healthy controls	1.538423/1.174544	1.3	0.01
HIV mono-infected vs Healthy controls	1.377158/1.174544	1.17	0.3452
HIV-TB coinfection vs HIV mono-infected	2.583382/1.377158	1.87	0.07
HIV-TB coinfection vs Pulmonary TB	2.583382/1.538423	1.67	0.15
Pulmonary TB vs HIV mono-infected	1.538423/1.377158	1.11	0.34

the mean densitometric intensities of C1QB for samples of pulmonary TB patients when compared to HIV mono-infected patients (p-value: 0.34). The expression of C1QB was found to be higher in HIV mono-infected patients compared to healthy humans, but the difference was not significant (p-value: 0.34) (Table 2).

4. DISCUSSION

Early and accurate diagnosis of TB in PLWH is challenging mainly due to the significant limitations in available diagnostics tests. Several studies have reported the upregulation of complement components in active TB disease [9, 15-17]. The present study investigated the abundance of C1QB protein in serum-derived exosomes of patients with HIV-TB coinfection, pulmonary TB, HIV mono-infected patients, and healthy humans. In the present study, the C1QB protein was found in serum exosomes of all study participants and no significant association was found between abundance of CIQB and demographic/clinical characteristics (gender, age, weight loss, smoking, and alcohol consumption habits etc) of the study participants. Previous studies also reported the presence of C1QB in exosomes derived from various body fluids in different disease conditions [18-20]. These findings indicated that the expression of C1QB in body fluid-derived exosomes might be consistent and exosomes can be used in liquid biopsy for C1QB analysis in different disease conditions.

The present study reports altered expression of C1QB in serum exosomes of study groups. The abundance of C1QB was found to be significantly higher in patients with HIV-TB coinfection and pulmonary TB compared to healthy humans; however, no significant difference was found when compared with HIV mono-infected patients. Similar to the present study, previous studies also reported higher expression of C1QB in peripheral blood/serum samples of active TB patients compared to individuals with latent TB infection and/or healthy humans [9, 10]. Since, C1QB is a key component of complement C1Q [21], the altered expression of C1QB can be directly related to the concentration of complement C1q in the body fluids of the host. Previously, Chai *et al*. (2014) investigated the complement gene expression in peripheral blood mononuclear cells of TB patients and controls using whole genome transcriptional microarray assays and reported that the expression of C1Q increases significantly in the peripheral blood of active TB patients compared to healthy humans and individuals with latent TB infection. Recently, significantly higher concentrations of C1Q have been reported in serum samples of TB patients from several geographically distinct regions, compared to healthy controls as well as individuals with latent TB infection, leprosy, pneumonia, and sarcoidosis [10]. In contrast to the present study, Li *et al*. (2020), using a series of bioinformatics analytical tools, reported significant upregulation of C1QB gene in HIV-TB patients as compared to cases with HIV/LTBI infection [22]. Previously, it has been observed that the expression of C1qA, C1qB, and C1qC genes is controlled in a similar manner; however the expression of C1QB was found to be upregulated upon IFNγ-stimulation as compared to C1QA and C1QC in macrophages

and dendritic cells [11]. Lu *et al*. (2008) also identified several toll-like receptor (TLR) ligands/ inflammatory cytokines responsible for increased (lipopolysaccharide, peptidoglycan, zymosan, lipoteichoic acid, IFN-γ, and IL-6) and decreased (LIL-1) production of C1q by macrophages or dendritic cells [23]. The variations in the findings of present study with others may be due to the small sample size and methodological differences. The small sample size and use of a single diagnostic test (western blotting) are the major limitations of the present study. Further cohort-based studies using advanced proteomic technologies (i.e. LC-MS/MS, MALDI-TOF, Protein Microarray) and more disease controls (i.e patients with diabetes or malnutrition, different types of respiratory infection etc.) are required to establish the diagnostic potential and clinical utility of C1QB in HIV-associated human TB.

5. CONCLUSIONS

The present study reports a higher abundance of C1QB in serum exosomes of patients with HIV-TB coinfection and pulmonary TB as compared to HIV mono-infected patients and healthy humans. Due to the evidence of increased concentration/ expression of C1QB in other disease conditions including HIV-associated diseases [18, 19, 20, 24], the clinical utility of C1QB as a crucial biomarker for the diagnosis of patients with HIV-TB coinfection as well as pulmonary TB seems to be limited. However, C1QB could be an 'adjunct biomarker' to discriminate TB patients from healthy humans. Further investigations are required to fully elucidate the impacts of altered expression of serum exosome-C1QB protein on the diagnosis and pathogenesis of TB in patients with or without HIV coinfection.

FUNDING

Authors are thankful to Indian Council of Medical Research, New Delhi for financial support (Grant number No.5/5/8/5/19/2014-ECD-I) and Department of Science and Technology, New Delhi for Woman Scientist Award to Dr. Rajbala Yadav (No SR/WOS-A/LS-129/2018).

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

1. WHO Report 2022, Global Tuberculosis Report.
2. Perkins, M. D. and Cunningham, J. 2007, J. Infect. Dis., 15(196), S15.
3. Yang, Q., Han, J., Shen, J., Peng, X., Zhou, L. and Yin, X. 2022, Medicine (Baltimore), 101(35), e30405.
4. Mekonnen, A. 2014, BMC Res. Notes, 7(1), 1.
5. Heesterbeek, D. A. C., Angelier, M. L., Harrison, R. A. and Rooijakkers, S. H. M. 2018, J. Innate Immun., 10(5-6), 455.
6. Cedzyński, M., Thielens, N. M., Mollnes, T. E. and Vorup-Jensen, T. 2019, Front. Immunol., 10, 1869.
7. Ostrycharz, E. and Hukowska-Szematowicz, B. 2022, Biomolecules, 12(2), 226.
8. Cliff, J. M., Lee, J. S., Constantinou, N., Cho, J. E., Clark, T. G., Ronacher, K., Ronacher, K., King, E. C., Lukey, P. T., Duncan, K., Van-Helden, P. D., Walzl, G. and Dockrell, H. M. 2021, J. Infect. Dis., 207, 18.
9. Cai, Y., Yang, Q., Tang, Y., Zhang, M., Liu, H., Zhang, G., Deng, Q., Huang, J., Gao, Z., Zhou, B., Feng, C. G. and Chen, X. 2014, PLoS One, 9(3), e92340.
10. Lubbers, R., Sutherland, J. S., Goletti, D., dePaus, R. A., Van-Moorsel, C. H. M., Veltkamp, M., Vestjens, S. M. T., Bos, W. J. W., Petrone, L., Del-Nonno, F., Bajema, I. M., Dijkman, K., Verreck, F. A. W., Walzl, G., Gelderman, K. A., Groeneveld, G. H., Geluk, A., Ottenhoff, T. H. M., Joosten, S. A. and Trouw, L. A. 2018, Front. Immunol., 9, 2427.
11. Chen, G., Tan, C. S., The, B. K. and Lu, J. 2011, J. Biol. Chem., 286, 34941.
12. Kushwaha, S., Yadav, R., Kusuma, D., Goel, A., Chauhan, D. S. and Singh, A. V. 2023, Curr. Proteomics, 20, 75.
13. Wu, Y., Deng, W. and Klinke, D. J. 2nd. 2015, Analyst., 140(19), 6631.
14. Bradford, M. M. 1976, Anal. Biochem., 72, 248.
15. Wang, C., Wei, L. L., Shi, L. Y., Pan, Z. F., Yu, X. M., Li, T. Y., Liu, C. M., Ping, Z. P., Jiang, T. T., Chen, Z. L., Mao, L. G., Li, Z. J. and Li, J. C. 2015, Sci. Rep., 5, 15615.

16. Jiang, T. T., Shi, L. Y., Wei, L. L., Li, X., Yang, S. U., Wang, C., Chang, M. L., Zhong, L. C., Hui, H. T., Zhong, J. L. and Ji, C. L. 2017, PLoS One, 12, e0173304.
17. Scriba, T. J., Penn-Nicholson, A., Shankar, S., Hraha, T., Thompson, E. G., Sterling, D., Nemes, E., Darboe, F., Suliman, S., Amon, L. M., Mahomed, H., Erasmus, M., Whatney, W., Johnson, J. L., Boom, W. H., Hatherill, M., Valvo, J., DeGroote, M. A., Ochsner, U. A., Aderem, A., Hanekom, W. A. and Zak, D. E. 2017, PLoS Pathog., 13, e1006687.
18. Barberis, E., Vanella, V. V., Falasca, M., Caneapero, V., Cappellano, G., Raineri, D., Ghirimoldi, M., De Giorgis, V., Puricelli, C., Vaschetto, R., Sainaghi, P. P., Bruno, S., Sica, A., Dianzani, U., Rolla, R., Chiocchetti, A., Cantaluppi, V., Baldanzi, G., Marengo, E. and Manfredi, M. 2021, Front. Mol. Biosci., 8, 632290.
19. Jia, X., Zhai, T. and Zhang, J. A. 2021, Front. Immunol., 12, 730089.
20. Meng, S., Wang, T., Zhao, Q., Hu, Q., Chen, Y., Li, H., Liu, C., Liu, D. and Hong, X. 2023, J. Proteome Res., 22(1), 123.
21. Beurskens, F. J., Van-Schaarenburg, R. A. and Trouw, L. A. 2015, Mol. Immunol., 68, 6.
22. Li, X., Xie, Y., Zhang, D., Wang, Y., Wu, J., Sun, X. and Wu, Q. 2020, Ann. Clin. Lab. Sci., 50(5), 600.
23. Lu, J., Teh, B. K., Wang, L., Wang, Y., Tan, Y. S., Lai, M. C. and Reid, K. B. 2008, Cell. Mol. Immunol., 5, 9.
24. Esmail, H., Lai, R. P., Lesosky, M., Wilkinson, K. A., Graham, C. M., Horswell, S., Coussens, A. K., Barry, C. E. 3rd, O'Garra, A. and Wilkinson, R. J. 2018, Proc. Nat. Acad. Sci. USA, 115(5), E964.