Differences of Total Activity and Heat Tolerance of Complement in Plasma Between Black and Yellow Populations

CURRENT STATUS: POSTED

Abdelhakam G. Tamomh
Dalian Medical University

Xiaojun Jin
Dalian Medical University

Hui Liu
Dalian Medical University

immunology@dlmedu.edu.cn Corresponding Author
ORCID: https://orcid.org/0000-0003-3974-0930

DOI: 10.21203/rs.2.19294/v1

SUBJECT AREAS
Biomedical Engineering

KEYWORDS
Mathematic Model, Total complement activity, Heat tolerance, Complement tolerance test, Kolmogorov-Smirnov Z test, Ethnic groups
Abstract

Background

The goal of this paper is to compare and evaluate the differences in total activity and Heat Tolerance of complement distribution Between Black and Yellow populations.

Methods

Blood samples of Black and Yellow healthy individuals were randomly collected, and plasma was obtained. The test plasma was diluted in a five series concentration, following with standard reaction and experimental reaction measurements. The basic principle of heat tolerance temperature calculation is to consider the ratio value (ratio method) in which heat tolerance of TCA calculated and plotted according to the area of trapezoidal and triangle as follows: $C_x^\circ C=OD1-OD5+(OD2-OD5+OD3-OD5+OD4-OD5)*2$. Where $C_x^\circ C$ is the total complement activity of the specimen measured in the two reaction conditions.

Results

The total activity of complement in Black individuals was statistically significantly lower than Yellow individuals ($P<0.05$). The heat tolerance of total complement activity was higher among blacks than yellow individuals with no differences between the two groups ($P>0.05$). The frequency values of total activity of complement distributed in the yellow individuals was statistically higher than Blacks ($P<0.05$) and The heat tolerance of total complement activity was statistically higher significant among Blacks than Yellow individuals ($P<0.05$).

Conclusions

There is a high differences in total complement activity and heat Tolerance distribution between Black and Yellow populations, may predict health status and innate immunity level differences between African race and Asian race.

Background

Black (African) and Yellow (Asian) populations environments, ethnic groups, and their geographical areas are more different, so innate and adaptive immune responses are different. Black populations may their immune responses strong against various diseases, and the strength might be result from
their innate immunity or adapting to local environments survival, thus resisting various pathogens or infections. Therefore, individuals of Black and Yellow populations are different in their sensitivity to diseases. To this end, we compare and explain the heat tolerance and total activity of complement in plasma using a new method between Black and yellow individuals. Complement is the first line of host immune defense system which involved in the elimination pathogens and dying cells of the host[1, 2]. Complement has been early described as an element key of innate immunity and has a major and great role in modulation of adaptive immunity [2, 3], and still need more attention.

A set of heat-sensitive glycoproteins that constitute a complement in serum of human has enzymatic activity [3-5]. In fact, complement is not one component or element. Complement also known as a complement system, which is composed from regulatory proteins, receptors and a solid part proteins, and these groups of proteins have biological features or functions [6, 7]. Complement solid part group in the body fluids is present for participation in the chain reaction process of activation. Complement activation and the biological role of active products can be regulated by Existing of complement as a membrane-bound form or the soluble form, and complement regulatory proteins[6, 8, 9]. Complement component proteins and their cognate receptors interaction has been explained to control or dominate several tasks ranging from activation of complement to differentiation and metabolism of adaptive immune cells[1-3].

Three activation pathways forms resulting from selectivity of complement which recognizes foreign pathogens and damaged or destroyed cells of itself; the classic, the alternative and lectin pathways[4, 10, 11]. The classic pathway is activated through antigen-antibody complexes interactions which cleaves C3. The alternative pathway is activated via formation of a complex between C3 and factor B, and especially the reaction occurs on the bacteria surface. The lectin pathway is activated by interaction between mannan-binding lectin (MBL) and certain carbohydrates, that are found only in special or certain bacteria[12-15]. All components of the complement are activated in a very slow speed in the plasma, that is a threat to the cell itself. Some serum or plasma and membrane proteins expressed by self cells for inhibit complement activation of body’s own system to protect or prevent itself from damage. Some types of membrane-bound proteins or plasma proteins are identified and
have been known which can inhibit the activation of complement to prevent or protect catastrophic consequences process after complement activation to their cells[11, 16-18]. Complement regulatory system act mainly to recognizes and regulates of some enzymes stability in activation pathways and inhibits or reduces activated complement production. Additionally, all cell surfaces explain or express three types of membrane proteins (as example, membrane cofactor protein(MCP), membrane inhibitor of reactive lysis (CD59) and decay accelerating factor (DAF)) to reduce and inhibit complement from activation themselves and protect from damaging or destroying their own as a result of complement mediation[3, 19, 20]. Complement dysfunction, deficiency or over activity is closely associated or related to the development and occurrence of various diseases[21-24]. Therefore, the determination of the complement total activity and it is sensitivity to heat is a great and important significance in assessing and evaluating immune responses and health status and in diagnosing or investigating various diseases. Most methods have been created and developed to analyzed and measure the total complement activity (TCA) (as example, CH50 method, single tube titration method, and liposome immunoassay[3, 25, 26], and no other studies estimate and measure the heat tolerance of total complement activity, even between black and yellow populations and no similar studies.

This present study attempted to become the foundation and establishment of a new detection system for heat tolerance of TCA to give new vision to the role of complement and open new direction in clinical laboratory analysis. The detection of heat tolerance of TCA will greatly develop and improve the rates of diagnosis and investigation of clinicians in complement related or associated diseases, thereby further assessing and evaluating the health and immune status of patients. This present study attempted to analyze and detect the total activity and heat tolerance of complement between Blacks and Yellow individuals as first compare study, to explore the differences and the role of innate and adaptive immunity between the two different ethnic populations.

Results
The total complement activity and heat tolerance of total complement activity parameters among Sudanese and Chinese are presented in Table 2. The total complement activity in the samples
obtained from Black individuals (Sudanese) was statistically significantly lower than that of in samples obtained from Yellow individuals Chinese (p < 0.05). The heat tolerance of total complement activity between Blacks and yellow groups was higher among blacks than yellow individuals with no statistically differences between the two groups (p > 0.05).

The difference in the degree of frequency distribution of total complement activity between the two groups was explained in Fig. 2 and Table 3. The frequency values of total complement activity distributed in the yellow individuals was statistically higher than Blacks (P < 0.05). The difference in the degree of frequency distribution in heat tolerance of total complement activity between the two groups was explained in Fig. 3 and Table 4. The frequency values distribution of heat tolerance of total complement activity was statistically higher significant among Blacks than Yellow individuals (P < 0.05).

Discussion

The values of total activity of and heat tolerance of complement measured and calculated among different ethnic groups may lead to open a new doors and give more attention for understanding the role of complement in host immune responses complexes to various diseases. Some individuals are different in their sensitivity to infections or diseases. Complement is a set of heat-sensitive components and has a very slow speed in plasma of humans[18, 24, 27]. Most and variety methods measure a TCA in serum or plasma, and no study measure or calculate the heat tolerance of TCA, even between Blacks and yellow individuals as different ethnic groups.

This study was aimed to determine and detect the total activity and heat tolerance of complement and compare its values between Sudanese and Chinese populations. The total activity of complement was measured according to the method which described and explained by Dong R and Liu H[3]. The heat tolerance of complement activity was measured using a new ratio method which consider the changing in ratio values as proposed in this paper Fig. 1.

The total complement activity and heat tolerance parameters together are essential parameters for describing the host immune responses and rarely used to describe or observe the changes in immune characteristics features of individuals among different ethnic groups as between Sudanese and
Chinese individuals or other similar researches or studies. Furthermore, the total activity and heat tolerance of complement in this present study can expect and predict the health immune status among different ethnic groups, or to obtain a new novel and great information on the role of complement in innate and adaptive immunity. In our present work, all subjects enrolled were healthy individuals from Sudanese and Chinese populations.

Our results expressed and showed that the total activity of complement was higher statistically among Chinese individuals than Sudanese individuals. The complement levels may vary resulting from the differences in state of innate or adaptive immunity of individuals[2, 28]. Further analysis revealed that the heat tolerance of complement activity was statistically higher among Sudanese individuals rather than Chinese individuals, but no significantly differences between the two populations. Clearly, a change in the total activity and heat tolerance of complement in different populations may result from the differences of their innate and adaptive immune responses[24, 28].

The present study indicates a clear differences in the calculated values of total activity and heat tolerance of complement between Sudanese and Chinese individuals Table 2. The relevance analysis using a two-sample Kolmogorov-Smirnov Z test results showed that the frequency values of total complement activity distributed among the Chinese individuals was statistically higher than that frequency values distributed among Sudanese individuals Fig. 2 and Table 3. A difference in Complement activity may results from the difference of some individuals in sensitivity to various diseases.

Our finding revealed that the degree of frequency values distribution of heat tolerance of total complement activity was statistically higher significant among Sudanese than Chinese individuals Fig. 3 and Table 4. Complement is an element which sensitive to heat, and the level of total activity and the heat tolerance of complement varies between individuals may express the state of various infections or diseases, and differences of individuals immune responses[1, 6, 7, 29, 30]. Accordingly, measuring and detecting the total activity and heat tolerance of complement between two ethnic groups, as Sudanese and Chinese populations, could expect or predict the health status or individuals immune status. In addition, improving or developing our detection or measurement system may help
for understanding the role of complement in innate and adaptive immune responses against various diseases, possibly leading to a better diagnosis an increase in the high efficacy of medicine, and health improvements. Other or further researches or studies should be applied to investigate complement components and assess the mechanism process of total activity and heat tolerance of complement to understand the immune characteristics features of individuals in various and different ethnic groups.

Conclusion
We conclude that, there was a high differences in total complement activity and heat Tolerance distribution between Black and Yellow populations, may expect the individuals health status, and in addition may explain and express the role and differences of innate and adaptive immunity among African race and Asian race.

Methods
Subject and Specimen:
A total of sixty-six blood samples of 39 Yellow (Chinese) (25 males and 14 females; mean age 30.77 ± 6.9 years ) and 27 Black (Sudanese) (20 males and 7 females; mean age 29.44 ± 4.07 years ) healthy individuals were randomly collected. Heat tolerance of total complement activity was analyzed and measured and calculated using a new ratio method. This study was approved by the Institutional Ethics Committee of Dalian Medical University.

Measurement of heat tolerance of TCA:
To measure the heat tolerance of total complement activity a complement tolerance test (CTT) was done. We replaced the traditional sheep red blood cells with human red blood cells as a hemolysis indicator system, and rabbit-anti-human red blood cell antibody was used as a hemolysin (which diluted using Phosphate buffer solution(PBS) to prepare a titer of 2) and then used instead of rabbit-anti-sheep red blood cell antibody in the CTT. The venous blood was withdrawn from an elbow vein using an EDTA-K2 anti-coagulation tube. The whole blood was immediately centrifuged at 2,500 r/min for 5 minutes, and blood cells and plasma were separated for use. The plasma of each concentration was aliquoted into two portions, each at least 200 µL, and incubated in standard temperature
(STD.temp) at 37 °C and in experimental temperature (Exp.temp) at 47 °C in a water bath for 30 minutes, respectively [3, 31].

Human red blood cell (Blood type O) suspension (2%) was prepared as follows. Human EDTA anticoagulated red blood cells (1 mL) were added to normal saline (10 mL) and mixed gently. The cells were centrifuged at 2,500 r/min for 5 minutes, and the supernatant was discarded. The remaining red blood cells were washed with 10 mL of normal saline and centrifuged to obtain a clear and transparent supernatant. Then, 10 mL of PBS buffer was added, and the mixture was centrifuged at 2,500 r/min for 5 minutes, and the supernatant was discarded. A total of 200 µL of packed red blood cells were resuspended in 9.8 mL of normal saline to prepare a 2% human red blood cell suspension.

Two of the samples of plasma to be tested were numbered respectively and consecutively in 1.5 mL tubes. The reagent was added to each tube according to Table 1.

The reagents were mixed, incubated at 37 °C in a water bath for 30 minutes, and centrifuged at 2500 r/min for 5 minutes. Then, 100 µL of supernatant from each tube was added to a 96-well microtiter plate, and the absorbance (OD value) was read at a wavelength of 542 nm.

Calculation Model:

The basic principle of the calculation model is to consider the ratio value (ratio method) in which heat tolerance of TCA calculated and plotted according to the area of trapezoidal and triangle. We propose a basic principle of the calculation model in Fig. 1.

The CTT was then calculated as follows:

\[ C_{x^c} = \text{OD}_1 - \text{OD}_5 + (\text{OD}_2 - \text{OD}_5 + \text{OD}_3 - \text{OD}_5 + \text{OD}_4 - \text{OD}_5)*2 \]  

This formula is calculated and plotted according to the area of trapezoidal and triangle. The trapezoid area is equal to \((\text{upper bottom} + \text{lower bottom}) \times \text{height} / 2\). The area of the triangle is equal to the base \times \text{height} / 2. The shadow area is equal to the area of the three small trapezoids plus a small triangle, which can also be understood as the sum of the area of four trapezoids, and the upper bottom of the last trapezoid is 0. That is \((\text{OD}_2 - \text{OD}_5 + \text{OD}_1 - \text{OD}_5) \times \text{height} / 2 + (\text{OD}_3 - \text{OD}_5 + \text{OD}_2 - \text{OD}_5) \times \text{height} / 2 + (\text{OD}_4 - \text{OD}_5) \times \text{height} / 2 + (\text{OD}_4 - \text{OD}_5) \times \text{height} / 2\). CTT uses the ratio method, so
the height does not affect it. The area is only related to the length of the bottom side, we only consider the effect of the length of the bottom in the calculation. The formula is as follows. The middle three data (the length of the bottom OD₂-OD₅, OD₃-OD₅ and OD₄-OD₅) are applied twice. For example, taking one Chinese sample as No. 1 at STD.temp 37 °C condition the calculation as bellow;

\[ C_{37\,^\circ\text{C} - \text{No.} \, 1} = OD_1 \cdot OD_5 + (OD_2 \cdot OD_5 + OD_3 \cdot OD_5 + OD_4 \cdot OD_5)^*2 \]

\[ = 0.114 - 0.041 + (0.112 - 0.041 + 0.100 - 0.041 + 0.081 - 0.041)^*2 \]

\[ = 0.413 \]

\[ \text{CTT} = \frac{C_{47\,^\circ\text{C}}}{C_{37\,^\circ\text{C}}} \]

where \( C_{x\,^\circ\text{C}} \) is the total complement activity of the specimen measured after 30 minutes in a water bath at \( x \) °C.

Statistical analysis

The heat tolerance of total complement activity among Sudanese and Chinese healthy individuals were described in quartile values, because of the non-normal distribution. Mann–Whitney U test was used to analyze the differences between the two group. The Degree of distribution in heat tolerance of total complement activity between the two groups assessed and evaluated using a two-sample Kolmogorov-Smirnov Z test. The calculation was performed using Windows software for Social Sciences (SPSS 21.0). Data were considered to be statistically significant when the probability of type I error was 0.05 or less.

Abbreviations

MBL:mannan-binding lectin; PBS:Phosphate buffer solution; MCP:membrane cofactor protein;

DAF:decay accelerating factor; TCA:total complement activity; CTT:complement tolerance test.

Declarations

**Authors’ contributions:**

AGT, XJ and HL were participated in the design of the study, performed the statistical analysis and AGT wrote the paper. XJ and AGT were performed the data acquisition and experiments. All authors read and approved the final manuscript.

**Acknowledgements**
Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of Dalian Medical University.

**Funding**

None.

**References**

1. Giang J, Seelen MAJ, van Doorn MBA, Rissmann R, Prens EP, Damman J. Complement Activation in Inflammatory Skin Diseases. Front Immunol. 2018; 9: 639.

2. Killick J, Morisse G, Sieger D, Astier AL. Complement as a regulator of adaptive immunity. 2018; 40:37-48.

3. Dong R, Liu H, Establishment of a method for measuring total complement activity based on a hemolysis system using own red blood cells. J Immunol Methods. 2016; 430:21-7.

4. Okroj M, Potempa J. Complement Activation as a Helping Hand for Inflammophilic Pathogens and Cancer. Front Immunol. 2018; 9:3125.

5. Gialeli C, Gungor B, Blom AM. Novel potential inhibitors of complement system and their roles in complement regulation and beyond. Mol Immunol. 2018; 102: 73-83.

6. Ekdahl KN, Persson B, Mohlin C, Sandholm K, Skattum L, Nilsson B. Interpretation of Serological Complement Biomarkers in Disease. Front Immunol. 2018; 9: 2237.
7. Gaya da Costa M, Poppelaars F, van Kooten C, Mollnes TE, Tedesco F, Würzner R, et al. Age and Sex-Associated Changes of Complement Activity and Complement Levels in a Healthy Caucasian Population. Front Immunol. 2018; 9: 2664.

8. El-Shamy A, Branch AD, Schiano TD, Gorevic PD. The Complement System and C1q in Chronic Hepatitis C Virus Infection and Mixed Cryoglobulinemia. Front Immunol. 2018; 9: 1001.

9. Chakraborty S, Karasu E, Huber-Lang M. Complement After Trauma: Suturing Innate and Adaptive Immunity. Front Immunol. 2018; 9: 2050.

10. Kim MY, Guerra MM, Kaplowitz E, Laskin CA, Petri M, Branch DW, et al. Complement activation predicts adverse pregnancy outcome in patients with systemic lupus erythematosus and/or antiphospholipid antibodies. Ann Rheum Dis. 2018; 77: 549-555.

11. Geller A, Yan J. The Role of Membrane Bound Complement Regulatory Proteins in Tumor Development and Cancer Immunotherapy. Front Immunol. 2019; 10:1074.

12. Łukawska E, Polcyn-Adamczak M, Niemir ZI. The role of the alternative pathway of complement activation in glomerular diseases. Clin Exp Med. 2018; 18: 297-318.

13. Dobó J, Kocsis A, Gál P. Be on Target: Strategies of Targeting Alternative and Lectin Pathway Components in Complement-Mediated Diseases. Front Immunol. 2018; 9: 1851.

14. Lilienthal GM, Rahmöller J, Petry J, Bartsch YC, Leliavski A, Ehlers M. Potential of Murine IgG1 and Human IgG4 to Inhibit the Classical Complement and Fcgamma Receptor Activation Pathways. Front Immunol. 2018; 9: 958.

15. Barkai LJ, Sipter E, Csuka D, Prohászka Z, Pilely K, Garred P, et al. Decreased Ficolin-3-mediated Complement Lectin Pathway Activation and Alternative Pathway Amplification During Bacterial Infections in Patients With Type 2 Diabetes Mellitus.
16. Schubart A, Anderson K, Mainolfi N, Sellner H, Ehra T, Adams CM, et al. Small-molecule factor B inhibitor for the treatment of complement-mediated diseases. Proc Natl Acad Sci U S A. 2019; 116: 7926-7931.

17. Haapasalo K, Meri S. Regulation of the Complement System by Pentraxins. Front Immunol. 2019; 10: 1750.

18. Chaturvedi S, Brodsky RA, McCrae KR. Complement in the Pathophysiology of the Antiphospholipid Syndrome. Front Immunol. 2019; 10: 449.

19. Kurtoğllu AU, Koçtekin B, Kurtoğlu E, Yildiz M. The effect of splenectomy on complement regulatory proteins in erythrocytes in beta-thalassemia major. Arch Med Sci. 2019. 15: 191-195.

20. Eldewi DM, Alhabibi AM, El Sayed HME, Mahmoud SAK, El Sadek SM, Gouda RM, et al. Expression levels of complement regulatory proteins (CD35, CD55 and CD59) on peripheral blood cells of patients with chronic kidney disease. Int J Gen Med. 2019; 12: 343-351.

21. Carpanini SM, Torvell M, Morgan BP. Therapeutic Inhibition of the Complement System in Diseases of the Central Nervous System. Front Immunol. 2019; 10: 362.

22. Schröder-Braunstein J, Kirschfink M. Complement deficiencies and dysregulation: Pathophysiological consequences, modern analysis, and clinical management. Mol Immunol. 2019; 114: 299-311.

23. Berentsen S, Hill A, Hill QA, Tvedt THA, Michel M. Novel insights into the treatment of complement-mediated hemolytic anemias. Ther Adv Hematol. 2019; 10: 2040620719873321.

24. Conigliaro P, Triggianese P, Ballanti E, Perricone C, Perricone R, Chimenti MS. Complement, infection, and autoimmunity. Curr Opin Rheumatol. 2019; 31: 532-541.
25. Tange CE, Johnson-Brett B, Cook A, Stordeur P, Brohet F, Jolles S, et al. Quantification of human complement C2 protein using an automated turbidimetric immunoassay. Clin Chem Lab Med. 2018; 56: 1498-1506.

26. Puissant-Lubrano B, Fortenfant F, Winterton P, Blanche A. A microplate assay to measure classical and alternative complement activity. Clin Chem Lab Med. 2017; 55: 845-853.

27. Tatomir A, Talpos-Caia A, Anselmo F, Kruszewski AM, Boodhoo D, Rus V, et al. The complement system as a biomarker of disease activity and response to treatment in multiple sclerosis. Immunol Res. 2017; 65: 1103-1109.

28. Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, Roumenina LT. Complement System Part II: Role in Immunity. Front Immunol. 2015; 6: 257.

29. Tortajada A, Gutierrez E, Pickering MC, Praga Terente M, Medjeral-Thomas N. The role of complement in IgA nephropathy. Mol Immunol. 2019; 114: 123-132.

30. Tamomh AG, Liu H. Differences in Antibodies Against Blood Group, HBV, and Salmonella Regarding Protein Content, Activity, and Affinity in Black and Yellow Healthy Individuals. Jundishapur J Microbiol. 2019; 12: e94687.

31. Xiaojun J, Hui L. An Angle Compared Index with Hybrid of Changes in the Ratio and Amplitude for Quantitative Evaluation of Disease Risk, Biological Function, and Biomarker Efficacy. Biomed Res Int. 2019; 2019: 8693719.

Tables

Table 1.

| Number                                      | 1   | 2   | 3   | 4   | 5   |
|---------------------------------------------|-----|-----|-----|-----|-----|
| 2% RBC suspension (μL)                      | 50  | 50  | 50  | 50  | 50  |
| 2U hemolysin (μL)                           | 50  | 50  | 50  | 50  | 50  |
| Specimen (μL)                               | 100 | 50  | 25  | 12.5| 0   |
| PBS buffer(μL) containing Ca\textsuperscript{2+} and Mg\textsuperscript{2+}(μL) | 800 | 850 | 875 | 887.5| 900 |
Table 2.

Relation of total activity and heat tolerance of complement in the two groups:

| Parameters       | Sudanese group |                  | Chinese group |                  | Z     | P     |
|------------------|----------------|------------------|---------------|------------------|-------|-------|
|                  | P25 | P50 | P75 |                  | P25 | P50 | P75 |
| Total activity   | 0.289| 0.313| 0.346| 0.311 | 0.350 | 0.379 | -2.420 | 0.016 |
| Heat tolerance   | 0.153| 0.251| 0.288| 0.165 | 0.199 | 0.227 | -0.659 | 0.510 |

Table 3.

Total activity of complement frequency distribution -K-S analysis between the two groups:

| Total activity | Mean | SD   | Kolmogorov-Smirnov Z | P   |
|----------------|------|------|----------------------|-----|
| Sudanese       | 0.317| 0.043| 1.491                | 0.023|
| Chinese        | 0.344| 0.049|                      |      |

Table 4.

Heat tolerance of complement activity frequency distribution-K-S analysis between the two groups:

| Heat tolerance | Mean | SD   | Kolmogorov-Smirnov Z | P   |
|----------------|------|------|----------------------|-----|
| Sudanese       | 0.228| 0.099| 1.661                | 0.008|
| Chinese        | 0.200| 0.044|                      |      |

Figures
Figure 1

Schematic diagram of ratio method for heat tolerance of TCA analysis.

Figure 2

Total activity of complement values frequency distribution among the two groups; A) Sudanese group; B) Chinese group.
Figure 3

Heat tolerance values frequency distribution among the two groups; A) Sudanese group; B) Chinese group.