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Elevated serum levels of decoy receptor 3 are associated with disease severity in patients with hemorrhagic fever with renal syndrome

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Abstract Hemorrhagic fever with renal syndrome (HFRS) is an acute viral infectious disease characterized by fever, hemorrhage and renal failure. HFRS has become a serious public health problem in China. Unfortunately, the pathogenesis of HFRS has not been completely clarified. The aim of this study is to investigate the changes of decoy receptor 3 (DcR3) and to further explore its potential roles in HFRS. The levels of serum DcR3 were measured by sandwich ELISA. We found serum DcR3 levels increased significantly, which reached peak value during the oliguric phase and in the critical group. Moreover, serum DcR3 levels were closely related to the levels of pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) and parameters reflecting kidney injury including BUN, creatinine (Cr) and proteinuria. This study indicates that high levels of serum DcR3 have associations with the disease stages, severity and degree of kidney damage. Meanwhile, our results suggest that DcR3 may play a dual role in HFRS pathogenesis. First, DcR3 is involved in the inflammatory cascade response resulting in capillary permeability and kidney injury in the early stage. Secondly, HTNV infection induced DcR3 expression at the convalescent phase may act as a feed-back mechanism in anti-inflammatory response. Thus, a study of DcR3 is essential for a better understanding of HFRS pathogenesis.

Keywords DcR3 · HFRS · TNF-α · HTNV

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Introduction

Hemorrhagic fever with renal syndrome (HFRS) is an acute viral infectious disease caused by Hantaan virus (*HTNV,* genus *Hantavirus,* family *Bunyaviridae*). Humans are mostly infected via inhaled aerosols of rodent excreta [1]. The course of HFRS is highly variable, ranging from asymptomatic to a lethal outcome [2]. HFRS is characterized by fever, hemorrhage and acute renal failure, with a mortality rate of ~5 %. Each year, 60,000–100,000 HFRS cases are reported worldwide, mostly from China [3, 4].

The pathogenesis of HFRS has not been completely clarified. HTNV infects endothelial cells, but does not have any direct cytopathic effects. However, the virus can induce dramatic changes in the barrier function of the vasculature integrity [5–7]. Activated lymphocytes result in the production of cytokines and inflammatory mediators, which contribute to the increased capillary permeability and kidney injury. It is believed that the immune system malfunction, rather than direct viral cytotoxicity, is the primary cause of vascular leakage and development of HFRS [8–11].

Decoy receptor 3 (DcR3) is a soluble protein belonging to the tumor necrosis factor (TNF) receptor superfamily [12, 13]. DcR3 can bind such ligands as FasL, LIGHT and TL1A, which are members of the TNF family [14–16]. DcR3 has pleiotropic immunomodulatory effects. It can suppress T cell chemotaxis, and regulate differentiation and

maturation of dendritic cells and macrophages. DcR3 also can down-regulate several co-stimulatory molecules [17–20]. Clinical data show that DcR3 can be detected in various diseases such as acute respiratory distress syndrome (ARDS,) inflamed intestinal epithelia, and systemic lupus erythematosus (SLE) [21–23]. HFRS is an infectious disease mediated by immune and inflammatory responses. Therefore, monitoring the levels of serum DcR3 in HFRS patients might aid in delineating the pathogenesis of HFRS.

In this study, we measured the levels of serum DcR3 in HFRS patients, and compared them in different phases and severities. Furthermore, the relationships between the levels of DcR3 and the levels of TNF-α, BUN, Cr, and proteinuria were analyzed. These results indicate that DcR3 is involved in the pathogenesis of HFRS.

Materials and methods

Patients

106 serum samples were randomly selected from inpatients with HFRS between 2010 and 2012 at Xi'an NO. 8 Hospital. The diagnosis of HFRS complied with the codes promulgated by the Ministry of Health of P.R.C. in 2008. There were 23 samples during the febrile phase, 20 samples during the hypotensive phase, 22 samples during the oliguric phase, 22 samples during the polyuric phase, and 19 samples were during the convalescent phase.

HFRS disease severity was evaluated by dividing patients into four groups according to their clinical symptoms and laboratory characteristics. The division criteria were as follows: (1) mild group ($n = 27$) temperature $\leq$39 °C, mild systemic poisoning symptoms; normal blood pressure and pulse pressure; small bleeding points in skin and mucous membranes but no significant bleeding in other areas; mild renal failure, proteinuria + ~++, in the obvious oliguric phase; (2) moderate group ($n = 27$) temperature 39–40 °C, apparent systemic poisoning symptoms, conjunctival edema; systolic blood pressure <12 kPa, or pulse pressure <3.47 kPa; bleeding in skin, mucous membranes or other areas; apparent renal failure, over +++ proteinuria, in the apparent oliguric phase; (3) severe group ($n = 30$) temperature $\geq$40 °C, serious systemic poisoning symptoms and exudation; systolic blood pressure <9.3 kPa, or pulse pressure <2.7 kPa, with shock symptoms; serious bleeding, such as skin ecchymosis and blood in body cavities; serious kidney damage, proteinuria to ++++, oliguria $\leq$5 days or anuria $\leq$2 days or in stages of disease overlap. If each of the previous three groups met more than 2 (including 2) aspects of the division criteria, diagnosis could be established; (4) critical group ($n = 22$) based on the criteria of the severe group, and with one of the following serious symptoms: refractory shock; serious bleeding in vital organs; significantly serious kidney damage, oliguria $\geq$5 days or anuria $\geq$2 days; or BUN $\geq$42.84 mmol/L (120 mg/dl); heart failure, pulmonary edema, acute respiratory distress syndrome; central nervous system complications, such as cerebral edema, cerebral hemorrhage and hernia; serious secondary infection; overlap of the hypotensive phase and the oliguric phase occurs.

Twenty blood donors in good health from the second Affiliated Hospital of Medical College were selected as normal controls.

The study protocol was approved by the ethics committee of our institution. Informed consents from all patients and healthy controls have been obtained before enrollment.

Sample collection

3 ml of fresh blood was collected into aseptic tubes containing coagulant from the vein of fasting patients in the morning. Blood samples were centrifuged at 3,000 rpm for 15 min. Sera were isolated, separated into Eppendorf tubes and stored at −80 °C before all assays. The entire process was completed within 2 h.

Enzyme-linked immunosorbent assay (ELISA) for detection of DcR3 and TNF-α

The levels of serum DcR3 and TNF-α were measured by double antibody sandwich ELISA kit (R&D Systems, USA) according to the manufacturer's instructions. All steps were undertaken at room temperature. For the assay, a 100 μl serum sample was added for each test. The OD values were read by a microtiter plate reader (BMG, polarstar optima) at 450 nm. The concentrations of DcR3 and TNF-α were calculated according to the standard curve. All measurements were conducted in triplicates.

Detection of BUN, Cr and proteinuria

Serum BUN and Cr levels were measured by the Olympus AU2700 automated biochemistry analyzer (Beckman Coulter, Mishima, Japan) as per the manufacturer's instructions. 24-h urine sample was collected from each inpatient and 3–5 ml sample was used to test. Proteinuria was measured by automated urine analyzer (DIRUI H-800, Changchun, China). Proteinuria was ranked into 6 grades according to the following criteria: –, 0–0.2 g/l; ±, 0.3–0.5 g/l; +, 0.5–1 g/l; ++, 1–3 g/l; +++, 3–5 g/l; ++++, 5–10 g/l.

Statistical analysis

For continuous variables, descriptive results were presented as mean ± SEM. The intergroup comparisons were made using one-way ANOVA for data that followed normal distribution. To assess the relationships between DcR3 and other variables, Pearson linear correlation analysis was used for variables that followed normal distribution and Spearman rank correlation analysis was used for those that did not. All analyses were performed with the SPSS 13.0 statistical software package. All tests were two-sided and a P value less than 0.05 was considered as statistically significant.

Results

The levels of serum DcR3 in different severity groups in patients with HFRS

The levels of serum DcR3 among different phases and normal controls in each severity group of HFRS patients were compared (Fig. 1a–d). We found that: (1) the levels of serum DcR3 at different clinical stages in mild, moderate, severe, and critical groups were higher than in normal controls ($P < 0.01$); (2) the levels of serum DcR3 increased initially from the febrile phase, reached peak value during the oliguric phase, and then decreased gradually until the convalescent phase in four different severity groups, respectively; (3) DcR3 levels during the convalescent phase in four groups were still elevated compared with those in normal controls, but lower than the levels during the febrile phase. Moreover, in the critical group, the levels of DcR3 sustained the highest level during the convalescent phase.

The levels of serum DcR3 at different phases in patients with HFRS

The levels of serum DcR3 among different groups and normal controls at each stage of HFRS were compared. Figure 2 shows that: (1) the levels of serum DcR3 are significantly higher in four different severity groups during

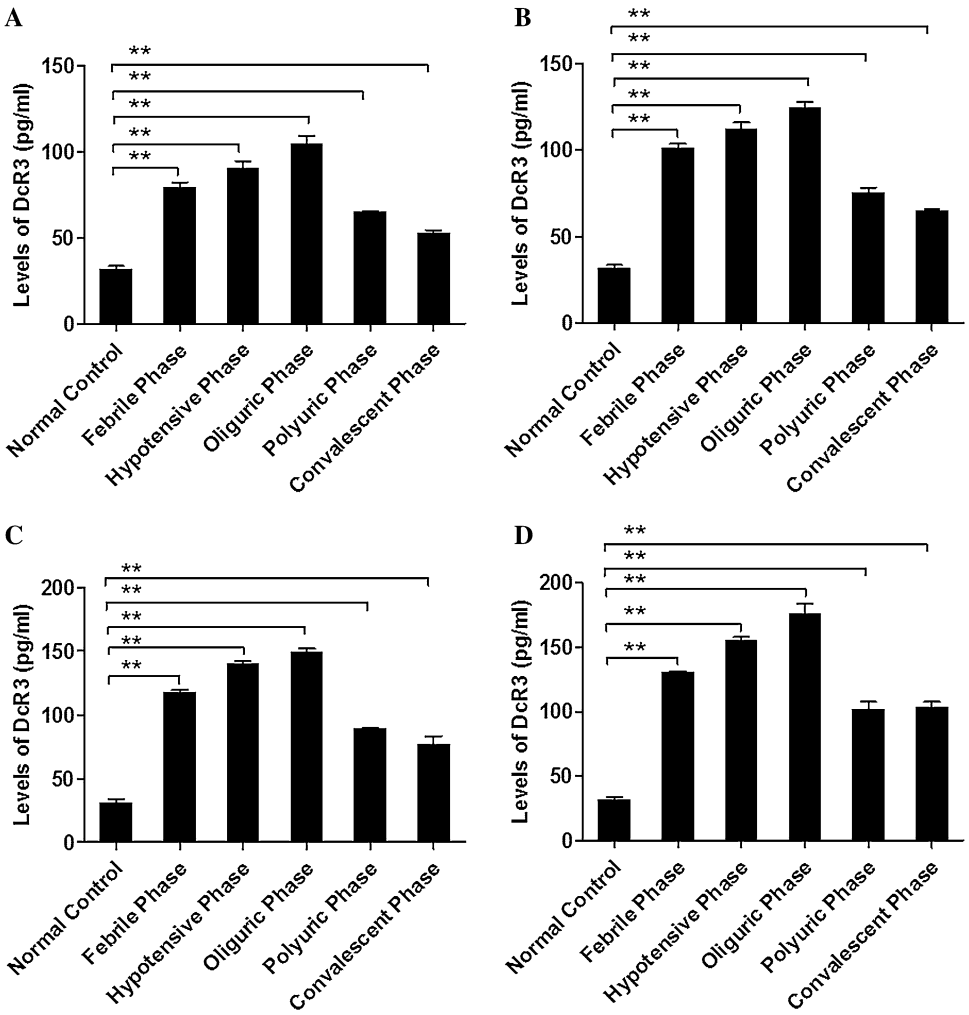

Fig. 1 Comparison of DcR3 among different phases and normal controls in each severity group. **a** Mild group, **b** moderate group, **c** severe group, and **d** critical group were investigated. There are five distinct phases in each group: febrile, hypotensive, oliguric, polyuric, and convalescent phases. The intergroup comparisons were made using one-way ANOVA for data that followed normal distribution. *Double asterisks* the difference is significant at the 0.01 levels. All measurements were conducted in triplicates

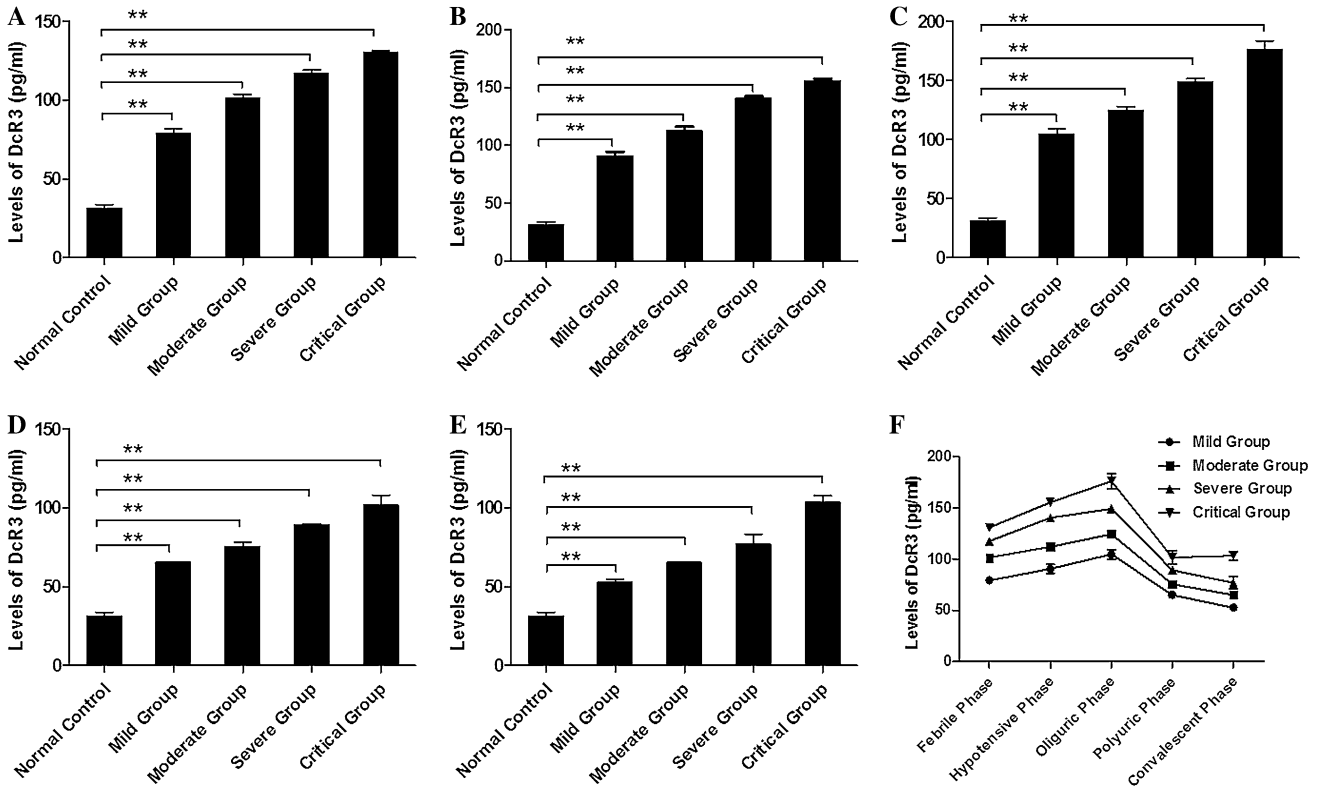

Fig. 2 Comparison of DcR3 among different groups and normal controls at each phase. **a** Febrile phase, **b** hypotensive phase, **c** oliguric phase, **d** polyuric phase, and **e** convalescent phase were investigated. **f** The dynamic changes of serum DcR3 at each stage in four different severity groups were analyzed. The intergroup comparisons were made using one-way ANOVA for data that followed normal distribution. *Double asterisks* the difference is significant at the 0.01 levels. All measurements were conducted in triplicates

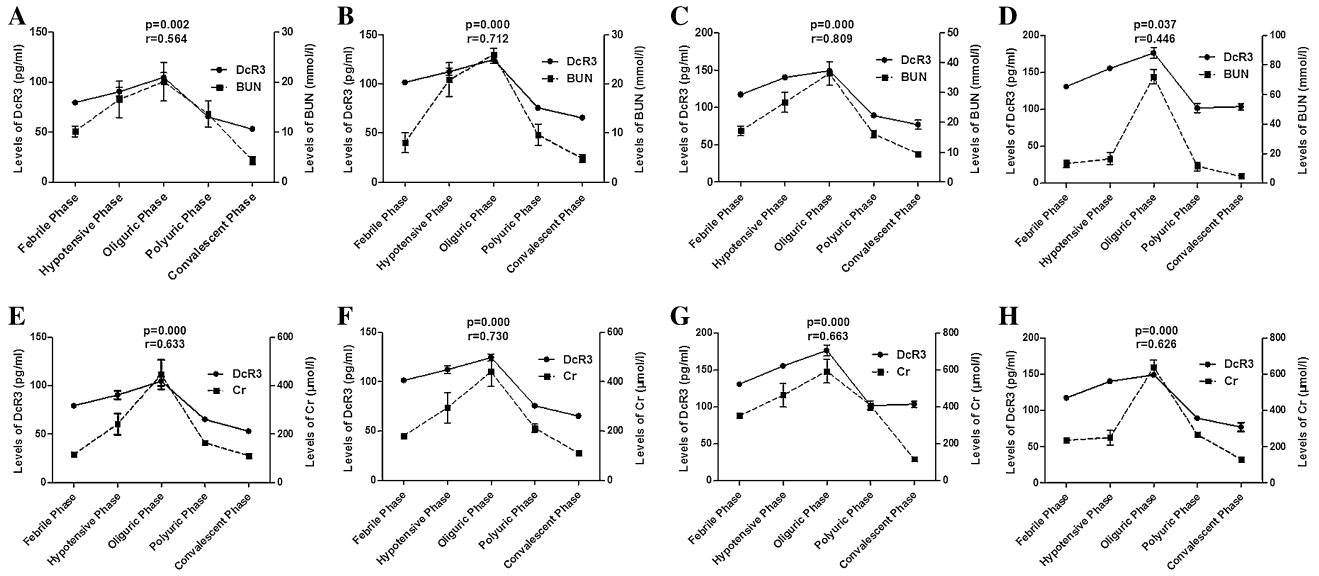

Fig. 3 Relationships between DcR3, BUN and Cr in four different severity groups at each stage. The levels of BUN in the **a** mild group, **b** moderate group, **c** severe group, and **d** critical group and the levels of Cr in the **e** mild group, **f** moderate group, **g** severe group, and **h** critical group were investigated, respectively. Pearson linear correlation analysis was used for variables that followed normal distribution. The correlation analysis was evaluated by correlation coefficient (r) and P value. P value less than 0.05 was considered as statistical significance. All measurements were conducted in triplicates

the febrile, hypotensive, oliguric, polyuric and convalescent phases than in normal controls ($P < 0.01$); (2) the levels of serum DcR3 increase significantly in an order from the slight group, moderate group, and severe group to the critical group at five phases (Fig. 2a–e); (3) the dynamic changes of serum DcR3 at each stage in four different severity groups show that the lowest levels of DcR3 are found during the convalescent phase in the mild group, whereas the peak levels are observed during the oliguric phase in the critical group (Fig. 2f).

Relationships between serum DcR3 levels and BUN, Cr and proteinuria levels

The levels of BUN and Cr significantly increased from the febrile phase, reached peak value during the oliguric phase, and then decreased up to the convalescent phase. In four different severity groups, the levels of BUN and Cr and the levels of DcR3 performed the almost consistent trends (Fig. 3a–h). Meanwhile, we analyzed the levels of serum DcR3 in different ranks of proteinuria in HFRS patients, and find that serum DcR3 levels increase significantly from low to high following an order from the rank "+", the rank "++" and the rank "+++" to the rank "++++". It shows that DcR3 levels reached the peak value in the rank "++++" (Fig. 4). Moreover, we find that there are positive correlations between the levels of DcR3 and the levels of BUN, Cr and proteinuria (Figs. 3, 4).

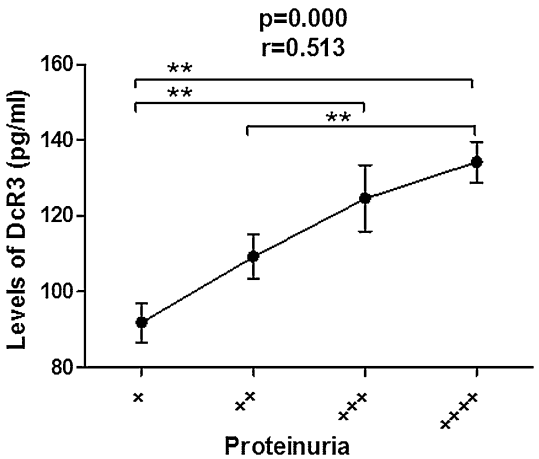

Fig. 4 Relationships between DcR3 and proteinuria. The levels of serum DcR3 in different ranks of proteinuria were analyzed. The intergroup comparisons were made using one-way ANOVA for data that followed normal distribution. *Double asterisks* the difference is significant at the 0.01 levels. Spearman rank correlation analysis was used for variables that not followed normal distribution. All measurements were conducted in triplicates

Correlation analysis between DcR3 and TNF-α level

The variation tendencies of DcR3 are consistent with TNF-α during different phases in four groups. Moreover, there is a positive correlation between the levels of DcR3 and the levels of serum TNF-α in different groups, respectively (Fig. 5).

Discussion

This study demonstrates that the levels of serum DcR3 are significantly elevated in patients with HFRS after HTNV infection. The data show that serum DcR3 levels increase initially from the febrile phase, reach peak value during the oliguric phase, and then decrease gradually up to the convalescent phase in four different severity groups. Moreover, the levels of serum DcR3 in patients with HFRS change from low to high following an order from mild group, moderate group, severe group to critical group during five phases. These findings suggest that the dynamic changes of serum DcR3 levels in HFRS are associated with the disease stage and severity.

Most notably, elevated serum DcR3 levels were still found during the convalescent phase in four different severity groups, especially in the critical group. However, clinically, the disease is almost recovered at this phase. Evidence shows that FasL-induced murine pulmonary inflammation is reduced by the intravenous pretreatment of a DcR3 analog [24], supporting a protective role of DcR3 in inflammation process. Hence, during the convalescent phase, DcR3 may participate in improving the repair of serious capillary permeability and kidney injury. High DcR3 expression during the convalescent phase may act as a feed-back mechanism in an anti-inflammatory response. Moreover, in the critical group, more DcR3 is required to work. It is conceivable that the decline and disappearance of DcR3 from serum could be regarded as the timescale of the disease.

Hence, DcR3 might play a dual role in the pathogenesis of HFRS. The activated lymphocytes in the kidneys of infected individuals may produce local or systemic inflammatory and immune responses, which might, in turn, contribute to the capillary leak syndrome and kidney damage characteristic of HFRS [11, 25, 26]. In our study, high serum DcR3 levels predict more serious outcomes in the early stage of HFRS, which appears to suggest an exacerbating effect of DcR3 on HFRS.

Our study also demonstrates that the levels of BUN and Cr significantly increase during the first four phases, and nearly return to normal during the convalescent phase. Moreover, the levels of serum DcR3 levels in HFRS patients are positively correlated with the levels of BUN,

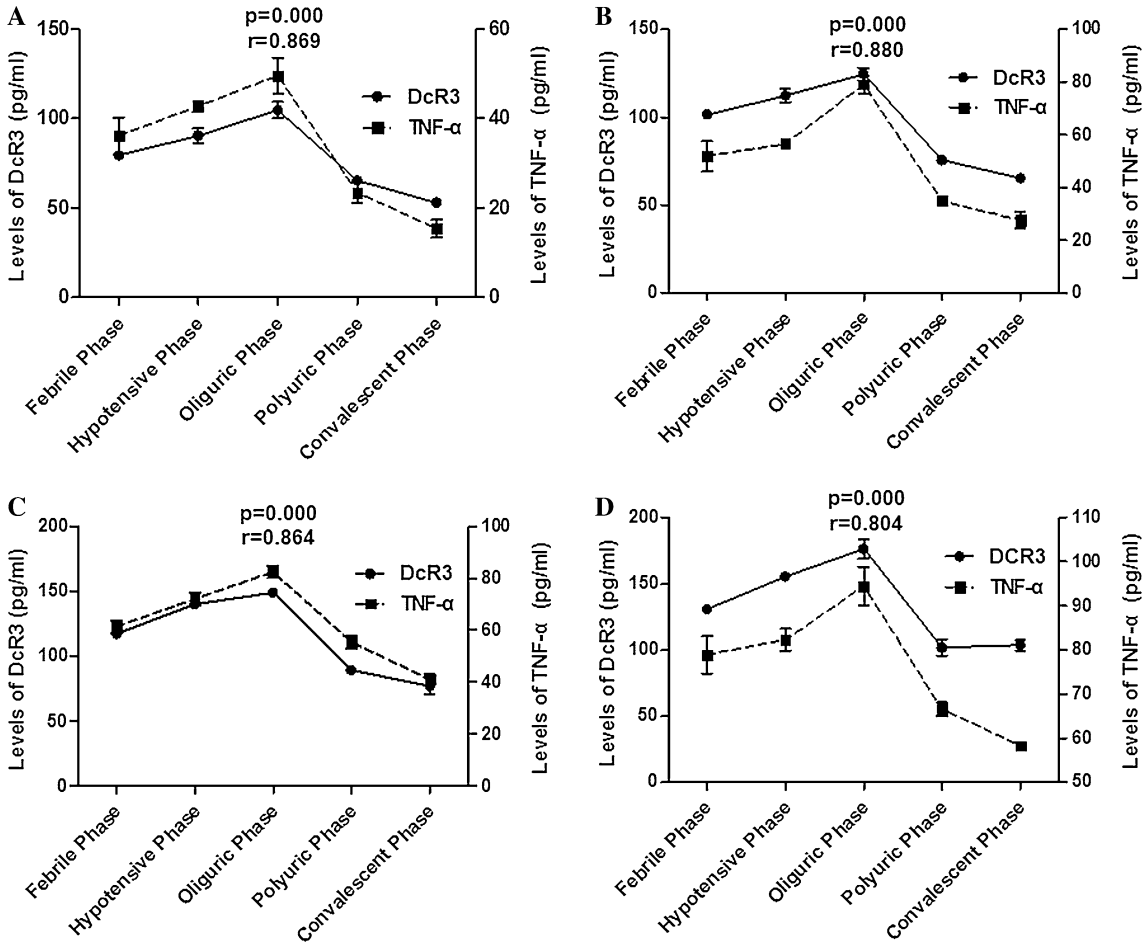

Fig. 5 Relationships between DcR3 and TNF-α. **a** mild group, **b** moderate group, **c** severe group, and **d** critical group were investigated. Pearson linear correlation analysis was used for variables that followed normal distribution. The correlation analysis was evaluated by correlation coefficient (*r*) and *P* value. *P* value less than 0.05 was considered as statistical significance. All measurements were conducted in triplicates

Cr and Proteinuria, which possibly reflect kidney function. Hence, high levels of DcR3 may reflect the status of kidney damage by HTNV infection. Clinically, the course of HFRS is highly variable, ranging from asymptomatic to a lethal outcome. Moreover, there are overlaps among the different phases and groups, which cause difficulties to clinicians for correct staging and typing of HFRS [2–4]. This study indicates that DcR3 may be a biomarker for patients with HFRS. First, DcR3 is closely related to the disease stage, severity and kidney injury. Moreover, DcR3 levels can be measured in accessible specimen (peripheral blood samples) by commercially available ELISA kits. Therefore, they have potential clinical application, and may well complement subjective judgments in diagnosis.

In our study, we also find that the variation tendencies of DcR3 are consistent with TNF-α at different phases in four groups. Moreover, DcR3 highly correlates with pro-inflammatory cytokines TNF-α, which may suggest that DcR3 is involved in the inflammatory cascade response in HFRS pathogenesis. Supporting this notion, DcR3 has been observed in various inflammatory and autoimmune diseases, such as ARDS with an infectious etiology, SLE and asthma [21, 23, 27]. Taking all these into consideration, we speculate that DcR3 may play important roles in the inflammatory and immune response in HFRS.

Meanwhile, high expression of DcR3 can also appear during influenza, measles and HFMD, not just in HFRS (data not shown). Therefore, the expression of DcR3 is also triggered by other acute viral infections.

In conclusion, the high levels of serum DcR3 have associations with the disease stages, severity and degree of kidney damage. This study indicates that DcR3 may be a potential biomarker for patients with HFRS. Moreover, we speculate that DcR3 may play a dual role in HFRS. First, DcR3 is involved in the inflammatory cascade response resulting in capillary permeability and kidney injury. Second, DcR3 probably contributes to the recovery from the disease during the convalescent phase.

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Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standard statement All human studies have been approved by the ethics committee of our institution and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent All persons gave their informed consents prior to their inclusion in the study.

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