Assessment Of Tumour Necrosis Factor-Alpha (Tnf-A) And Creatinine Levels In *Echis Ocellatus* Bite Victims In Jos Metropolis, Nigeria

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doi: 10.19044/esj.2016.v12n21p70 URL:http://dx.doi.org/10.19044/esj.2016.v12n21p70

Abstract

This study was designed to assess tumour necrosis factor-alpha and creatinine levels in *Echis ocellatus* bite victims. A total of 50 subjects were recruited. Out of this number, 40 were victims of *E. ocellatus* bite and the remaining 10 were non-victims of snake bite who served as the control group. Blood samples were collected from the victims within 24 hours of the snake bite and EchiTAb-G antivenom administered within the same period. Another batch of blood sample was collected 48 hours post-administration of the anti-venom. Tumour necrosis factor-alpha (TNF-alpha) levels were estimated by the Enzyme Linked Immunosorbent Assay technique while creatinine levels were determined using kinetic-spectrophotometric procedure. The mean serum levels of tumour necrosis factor-alpha and creatinine were significantly increased in *E. ocellatus* bite victims compared

with the control group (P<0.05). Furthermore, the mean serum level of TNF-alpha was significantly lower in *E. ocellatus* bite victims, post-administration of anti-venom, compared with the pre-administration of anti-venom (P<0.05). In contrast, no significant difference was observed in the mean serum level of creatinine in *E. ocellatus* bite victims, post-administration of anti-venom, compared with the pre-administration of anti-venom (P>0.05). Moreover, the mean serum level of creatinine was found to be significantly increased in *E. ocellatus* bite victims, post-administration of anti-venom, compared with the control group (P<0.05), while no significant difference was observed in the mean serum level of tumour necrosis factor-alpha in *E. ocellatus* bite victims, post-administration of anti-venom, compared with the control group(P>0.05). A positive correlation existed between tumour necrosis factor-alpha and creatinine levels in *E. ocellatus* bite subjects (r= 0.782). Echis ocellatus bite is a risk factor for renal damage indicated by an elevated serum creatinine, thus health authorities should make EchiTAb-G anti-venom freely available in health facilities and administered as quickly as possible to reduce the risk of renal damage in Echis ocellatus bite-prone areas.

Keywords: Tumor necrosis factor alpha, creatinine, Snake venoms, anti-venoms, Echis ocellatus

Introduction

Snakebite envenoming comprises a major public health problem among communities of the savanna region of West Africa, notably in Benin, Burkina-Faso, Cameroon, Ghana, Nigeria and Togo (Chippaux *et al*., 2007). Four families of venomous snakes are found in Nigeria--Viperidae, Elapidae, Colubridae and Actraspididae but three species carpet viper (*Echis ocellatus*), black-necked spitting cobra (*Naja nigricollis*) and puff adder (*Bitis arietans*), belonging to the first two families, are the most important snakes associated with envenoming in Nigeria, with the saw-scaled or carpet viper (*Echis ocellatus*) as the most important cause of snakebite mortality and morbidity in the region (Habib *et al.*, 2001). Envenomation by snakes of the family Viperidae is characterized by prominent local effects, including necrosis, hemorrhage, edema, and pain, which develops rapidly after the accident and often result in permanent sequelae (Warrell, 1995) and systemic alterations such as hemorrhage, coagulopathy, shock, and acute renal failure, may occur. Both local and systemic effects of these snake venoms have been associated with the action of a variety of venom components, notably metalloproteinases (Gutierrez and Lomonte, 1997).

Tumor necrosis factor- alpha (TNF-α) is a proinflammatory cytokine produced mainly by activated macrophages or monocytes and plays an

important role in diverse cellular events, such as the production of other cytokines, cell proliferation, differentiation and apoptosis (Beyaert and Fiers, 1994). The cellular changes in response to TNF-α are cell-type dependent. For example, TNF-α may modify the anticoagulant properties of endothelial cells, promote T cell proliferation, cause bone resorption, and induce the release of other inflammatory cytokines in many different cells.

TNF-α is not usually detectable in healthy individuals, however, elevated serum and tissue levels are found in inflammatory and infectious conditions (Robak *et al.*, 1998) and serum levels correlate with the severity of infections. Inappropriate production of TNF-α has been implicated in the pathogenesis of both acute and chronic inflammatory diseases such as septic shock, AIDS, arthritis and cancer (Tracey and Cerami, 1993).

Creatinine (MW 113 D) is the cyclic anhydride of creatine that is produced as the final product of decomposition of phosphocreatine. Creatine is synthesized in the kidneys, liver, and pancreas, primarily in the liver from arginine, glycine, and methionine and then transported to other tissues, such as muscle, where it is converted to phosphocreatine, which serve as high-energy source. Creatine phosphate loses phosphoric acid and creatine loses water to form creatinine, which passes into the plasma. Plasma creatinine concentration is a function of relative muscle mass and renal function (Bishop *et al.*, 2005). It remains within the reference interval until significant renal function has been lost.

This study was therefore designed to assess tumor necrotic factor alpha and renal damage in *Echis ocellatus* bite victims.

Materials and methods

Study sites

This research was carried out at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra state, Nigeria and Jos University Teaching Hospital Comprehensive Health Centre Zamko, Plateau State, Nigeria.

Study population

A total of 50 individuals were recruited for this study which included 40 *Echis ocellatus* bite subjects and 10 non-victims of snake bite which served as the control group. Samples were collected prior to anti-venom administration and 48 hours post-administration of the anti-venom from the victims of *Echis ocellatus* bite.

Exclusion criteria

Patients with renal disorders, inflammatory and autoimmune diseases such as Systemic lupus erythematosus (SLE), Scleroderma, Sjögren's

syndrome, Mixed connective tissue disease, Polymyositis/dermatomyositis and Rheumatoid arthritis were excluded in the study.

Ethical consideration and informed consent

The ethical approval for this research was obtained from the ethics committee of the Faculty of Health Science and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus and informed consent of the subjects were obtained.

Collection of samples

About 5ml of venous blood was collected aseptically from each subject and dispensed into a plain container for the determination of tumour necrosis factor-alpha and creatinine levels. The samples were centrifuged at 5,000 rpm for 5 minutes and the serum separated.

Analysis of parameters

The method employed in the estimation of tumour necrosis factor alpha level was an enzyme immunosorbent assay as described by Meade *et al* (1986) while plasma creatinine level was estimated using kinetic Jaffe reaction as described by Lipitskaia *et al*. (1989).

Statistical analysis

Statistical package for Social sciences (SPSS) version 20 was used for analysis using the students t-test and Pearson correlation. Values were deemed significant if $p < 0.05$

Results

The mean serum level of tumour necrosis factor-alpha in Echis ocellatus bite victims, pre-administration of anti-venom was significantly increased compared with the control subjects ($p < 0.05$). Similarly, a significant increase was observed in the mean serum level of creatinine in Echis ocellatus bite victims, pre-administration of antivenom, compared with the control subjects ($p < 0.05$) (Table 1).

There was a significant decrease in the mean serum level of tumour necrosis factor-alpha (TNF-α) post-administration of anti-venom, compared with the pre-administration of anti-venom, in the Test subjects ($p < 0.05$). Statistically no significant difference existed in the mean serum level of creatinine post-administration of anti-venom, compared with pre-administration of anti-venom, in the Test group ($P > 0.05$) (Table 2).

There existed no significant difference in the mean serum level of tumour necrosis factor-alpha in Echis ocellatus bite victims, post-administration of anti-venom, compared with the control subjects ($P > 0.05$).

However, a significant increase was observed in the mean serum level of creatinine in Test group, post-administration of antivenom, compared with the control subjects ($p < 0.05$). (Table 3)

In figure 1, a positive correlation was observed between tumour necrosis factor-alpha and creatinine levels in Echis ocellatus bite victims pre-administration of antivenom (r= 0.782).

Table 1: Comparison of tumour necrosis factor-alpha (TNF-α) and creatinine levels in control group and *Echis ocellatus* bite victims (Test group) pre-administration of anti-venom.

GROUPS	N	Tumour Necrosis factor (ng/ml)	Creatinine (µmol/l)
Echis ocellatus bite victims	40	42.87 ± 42.22	106.39 ±12.25
Control subjects	10	6.39 ± 10.12	85.06 ± 17.67
P-value		0.02*	0.01*

Table 2: Comparison of tumour necrosis factor-alpha and creatinine levels in pre and post-administration of anti-venom in the Test group.

GROUP	N	Tumour Necrosis factor (ng/ml)	Creatinine (µmol/l)
Pre-treatment	40	42.87 ± 42.22	106.39 ± 12.25
Post-treatment	10	18.68 ± 31.38	101.49 ± 17.93
P-value		0.02*	0.16

Table 3: Comparison of tumour necrosis factor-alpha (TNF-α) and creatinine levels in control subjects and *Echis ocellatus* bite victims post-administration of anti-venom.

GROUP	N	Tumour Necrosis factor (ng/ml)	Creatinine (µmol/l)
Post-treatment	40	18.68 ± 31.38	101.49 ± 17.93
Control	10	6.39 ± 10.12	85.06 ± 17.67
P-value		0.17	0.04*

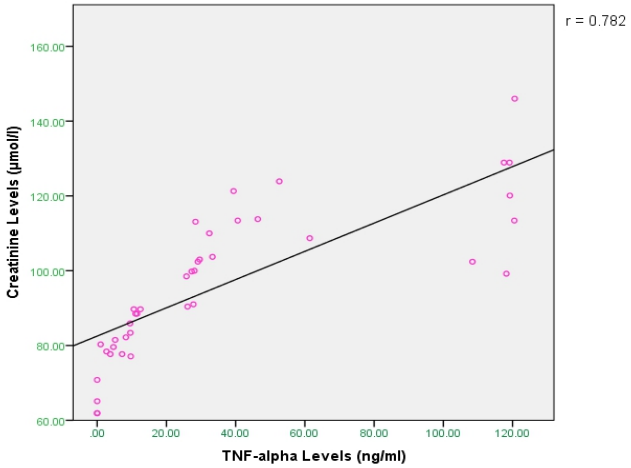

Fig 1: Correlation of tumour necrosis factor-alpha and creatinine levels in Echis ocellatus bite victims pre-administration of antivenom.

Discussion

Snakebite envenomation is a neglected tropical disease that affects each year, hundreds of thousands of individuals in tropical and sub-tropical areas of the world (Kasturiratne *et al*., 2008). In the north-eastern and central parts of Nigeria, Carpet viper (*Echis ocellatus*) along with other true and pit vipers, is the venomous snake responsible for most deaths in snakebite cases. Carpet viper venom induces a pathophysiological picture, characterized by immediate and prominent local tissue damage (myonecrosis, edema, inflammation, haemorrhage and hypovolemic shock) and coagulation disorders (defibrination) which might end in acute renal failure (Amoral *et al.*, 1985).

The parenteral administration of animal-derived anti-venoms constitutes the mainstay in the therapy of snakebite envenoming (WHO, 2010). In Nigeria, EchiTAb-G (a monospecific antivenom manufactured by Micropharm) developed against the venom of the Nigerian snake (*Echis ocellatus*) is used in the management of envenoming by this specie (Abubakar *et al*., 2010). Restoration of blood coagulability has been used as a surrogate marker of antivenom effectiveness in many clinical studies of viper-bite-induced consumption coagulopathy (Viser *et al.*, 2008). In this study, we assessed the capacity of *Echis ocellatus* venom to cause inflammation and/or renal damage and the efficacy of ECHITab-G in neutralizing these inflammatory and nephrotoxic effect of the snake venom. The mean serum level of tumour necrosis factor-alpha was significantly increased in *E. ocellatus* bite victims pre-administration of anti-venom, compared with the control group ($P<0.05$). Similarly, a significant increase was observed in the mean serum level of creatinine in *E. ocellatus* bite victims pre-administration of anti-venom, when compared with that of the control ($P<0.05$). Crocker *et al* (2010) and Moreira *et al* (2012), had earlier reported elevated plasma concentrations of TNF-α and other pro-inflammatory cytokines such as IL-6, IL-8 in envenomed humans. TNF is produced predominantly by activated macrophages and T lymphocytes as a 26 kDa protein, pro-TNF, which is expressed on the plasma membrane, where it can be cleaved in the extracellular domain by the matrix metalloproteinases, resulting in the release a soluble active 17 kDA form (Wang et al., 2003). Moura-da-Silva *et al*(1996) have shown that two venom zinc metalloproteinases (jararhagin from Bothrops jararaca venom and a metalloproteinase from Echis pyramidum leakeyi venom) successfully cleaved the recombinant glutathione-S-transferase-tumor necrosis factor-alpha fusion protein (GST-TNF-alpha) substrate to form biologically active TNF-alpha. This cytokine may contribute to the local necrosis and also induce the production of endogenous matrix metalloproteinases, which in turn generate a positive feedback mechanism resulting in continued cleavage

of pro-TNF-alpha. These circumstances may be the cause of the elevated plasma concentration of TNF-alpha.

The elevations in creatinine level in the test subjects, pre-administration of antivenom, observed in the present study are similar to that reported by Kale and Lonkar (2013), where the creatinine level was found to be 2.6 times the level of the control subjects. Muhammad (2009) also demonstrated significantly increased levels of serum creatinine in Guinea pigs envenomated with *Echis coloratus* and noted that this crude venom caused hepatic and renal dysfunction in the Guinea pigs. Furthermore, Gutie´rrez and Ownby (2003) and Montecucco *et al* (2008) reported that the venom of vipers contain myotoxic PLA2s and PLA2 homologues which induce rapid alterations to the plasma membrane of the muscle cells (myotoxicity), followed by irreversible cell injury. The myotoxic activity of the venom produces severe skeletal and cardiac muscle injury leading to myalgia and rhabdomyolysis with the subsequent release of myoglobin from damaged skeletal muscle into serum leading to myoglobinemia with further myoglobinuria which can result in damage to the kidneys (nephrotoxicity) as a result of myoglobin accumulation in the renal tubules. This is coupled with hypotension and may lead to acute renal failure - the cause of death among patients surviving to the early effects of snakebites (Markus *et al.*, 2012). Zhou *et al* (2008) also included direct nephrotoxicity of the snake venom, circulatory collapse, intravascular hemolysis and microangiopathic hemolytic anaemia as other contributing factors that could lead to acute renal disease observed in snake envenomation.

Furthermore, this study observed a significantly decreased mean serum level of tumour necrosis factor-alpha in Echis ocellatus bite victims, post-administration of antivenom, compared with the pre-administration of antivenom. However, there was no significant difference in the mean serum level of creatinine in Echis ocellatus bite victims, post-administration of antivenom compared with the pre-administration of antivenom ($P>0.05$).

Juan *et al* (2010), while assessing the immunological reactivity of EchiTAb-Plus-ICP, revealed a complete immunodepletion of the majority of venom components, including metalloproteinases, serine proteinases, C-type lectin-like proteins, some phospholipases A2 and L-amino acid oxidase.

In contrast, Stone *et al* (2013) found that after treatment with antivenom in Sri Lankan viper envenoming there were further increases in TNF-α and other cytokines(IL-6, IL-10), though, typical hypersensitivity reactions to antivenom occurred in 64% of the patients which correlated with severity of hypersensitivity reactions and strongly suggested that the increase reflects an immune response to the antivenom.

In this work, no significant difference was observed in the mean serum level of creatinine in the test group, post-administration of antivenom,

compared with the pre-administration of antivenom. Thein-Than *et al* (1991) had earlier reported that renal damage can develop very early and even when the patient arrives at hospital soon after the bite, the damage may already have been done. They further observed that even when anti-snake venom was administered within 1-2 hours after the snake bite, it was incapable of preventing acute renal failure. To buttress this, Warrell (1999) maintained that a victim in renal failure is evidence of the previous action of venom either directly on the kidney or by fibrin deposition and does not necessarily imply that the victim currently has un-neutralised venom in the system. Hence, Shastry *et al* (1977) suggested that declining renal parameters in snake bite victims require referral to a specialist nephrologist where either peritoneal dialysis or haemodialysis could be performed.

Statistically, no significant difference was observed in the mean serum level of tumour necrosis factor-alpha in *E. ocellatus* bite victims, post administration of antivenom, compared with the control group while a significant increase was observed in the mean serum level of creatinine in *E. ocellatus* bite victim, post-administration of antivenom compared with the control group. This futher illustrates the capacity of the anti-venom in neutralizing the inflammatory effects of the snake venom and its inability to reverse renal damage already caused by the snake venom.

A positive correlation was observed between tumour necrosis factor-alpha and creatinine levels in *E. ocellatus* bite victims, pre-administration of antivenom (r=0.782). It has been recorded that in addition to the hemorrhagic effect of SVMP, they are also involved in the pathogenesis of myonecrosis that could lead to increased renal damage and inflammatory reactions releasing inflammatory cytokines like TNF-alpha (Moura-da-Silva *et al.*, 1996). This implicates the action of snake venom metal as the major cause of this positive correlation observed between tumour necrosis factor-alpha and creatinine levels.

Conclusion

In this study, we have been able to show that tumour necrosis factor-alpha and creatinine levels were significantly increased in *Echis ocellatus* evenomation. This implies that Echis ocellatus evenomation is associated with alterations in renal function markers and inflammatory mediators. Thus envenomed subjects are at higher risks of acute kidney impairment.

Recommendations

Baseline and post assessment of the inflammatory and renal status of Echis ocellatus bite victims is recommended. EchiTAb-G antivenom should be made freely available in Echis ocellatus bite-prone areas and administered as quickly as possible to reduce the risk of renal damage associated with

Echis ocllatus evenomation.

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