

Clinical Diagnosis, Management and Microbiological Profiling of Necrotizing Soft Tissue Infections – A Prospective Study of North India Region

Tanya Bhalla¹, Amandeep Singh Nar^{1*}, Ravinder Pal Singh¹, Rohit Jindal², Anuj Bhalla¹, Atul Mishra¹

¹Department of Surgery, Dayanand Medical College and Hospital, Ludhiana

²Department of Surgical Oncology, SMS Medical College and Hospital, Jaipur

*Corresponding author:
Dr. Amandeep Singh Nar
Associate Professor
Department of Surgery
Dayanand Medical College
and Hospital, Ludhiana
Tel :- +919872019770
E-mail: amandeepnar@yahoo.com

Rezumat

Diagnosticul clinic, managementul și profilul microbiologic al infecțiilor necrozante de țesuturi moi - un studiu prospectiv pe populația din Nordul Indiei

Context: Infecțiile necrozante ale țesuturilor moi (INTM) sunt boli rapid progresive, potențial fatale, caracterizate prin necroza țesuturilor moi. Boala este extrem de letală în absența tratamentului. Cu toate acestea, diagnosticarea și gestionarea sa precoce pot duce la o scădere semnificativă a mortalității, salvând viața pacientului și prevenind pierderea membrilor; dar diagnosticul acestei afecțiuni în stadiu incipient este dificil. Prin urmare, lucrarea de față descrie pas cu pas un protocol pentru diagnosticarea precoce a INTM și aplicarea acestuia pentru gestionarea cu succes a INTM (tipul III) în populația din nordul Indiei.

Metode: Acest studiu prospectiv a fost realizat pe o perioadă de 1 an și 9 luni (decembrie 2017 -septembrie 2019) într-un singur centru terțiar (Colegiul și Spitalul Medical Dayanand, Ludhiana, Punjab, India) pe 115 pacienți diagnosticați cu INTM. Au fost înregistrate date demografice și clinice. După resuscitarea inițială, s-a practicat debridarea sau amputarea, iar probele colectate au fost supuse analizei microbiene și testelor de sensibilitate la antibiotice. Pe baza rezultatelor, a fost instituit un tratament farmacologic adecvat pentru a minimiza riscul de mortalitate și a grăbi recuperarea pacientului.

Rezultate: Acest studiu a arătat că, în populația selectată, infecțiile monomicrobiene gram negative sunt mai frecvente, cu atât mai mult, în rândul bărbaților cu diabet zaharat. Agentul patogen predominant s-a dovedit a fi *Escherichia coli* (40% din cazuri).

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Pacienții înrolați au avut INTM în stadiul 1, în absența sepsisului, ceea ce înseamnă că au solicitat asistență medicală în stadiile incipiente ale bolii. Debridarea sau amputarea au avut ca rezultat o scădere semnificativă a ratei mortalității (10,4%), chiar și în cazul INTM letale de tip III.

Concluzie: Abordarea prezentată poate ajuta la detectarea și gestionarea precoce a INTM, facilitând astfel salvarea vieții și a membrilor pacientului.

Cuvinte cheie: infecții necrozante ale țesuturilor moi, mortalitate, management precoce, fascie necrozantă, debridare, profilul microbian

Abstract

Background: Necrotizing Soft Tissue Infections (NSTIs) are rapidly progressive, potentially fatal illnesses characterized by necrosis of soft tissue. The disease is highly lethal if left untreated. However, its early diagnosis and management can result in significant decrease in mortality, saving the life of patient and preventing limb loss ; but its diagnosis in early stage is difficult. Therefore, the present work describes a step-by-step protocol for early diagnosis of NSTIs and its application for successful management of NSTIs (type III) in the North Indian population.

Methods: This prospective study was conducted over a period of 1 year and 9 months (December 2017 to September, 2019) in a single tertiary center (Dayanand Medical College and Hospital, Ludhiana, Punjab, India) with 115 patients suffering from NSTIs. Demographic, symptomatic and clinical features were recorded. After initial resuscitation, debridement or amputation was done and collected samples were subjected to microbial profiling and drug sensitivity tests. Based on results, suitable pharmacological treatment was started to minimize the risk of mortality and fasten the recovery of patient.

Results: The present work showed that in the selected population, monomicrobial gram negative infections are more prominent, more so, among diabetic males. The most prominent pathogen was found to be *Escherichia coli* (40% of cases). Enrolled patients had NSTIs in stage 1 and had no blood infection which means they sought medical attention in the early stages of the disease and the clinician was able to detect it. Debridement or amputation resulted in a significant decrease in the mortality rate (10.4%) even when the selected population had lethal Type III NSTIs.

Conclusion: The presented approach can aid in early detection and management of NSTIs, thus, helping in saving patient's life and limb.

Key words: necrotizing soft tissue infections, mortality, early management, necrotizing fasciitis, debridement, microbial profiling

Introduction

Necrotizing Soft Tissue Infections (NSTIs) are characterized by necrosis of soft tissue usually associated with rapidly progressive sepsis and shock. On the basis of monomicrobial or polymicrobial origin, NSTIs have been classified into the following types (1):

- Type I – polymicrobial infections, the most common form, causative organisms include a combination of gram positive

cocci, gram negative rods and anaerobes.

- Type II – monomicrobial infections, typically but not necessary to be caused by “flesh eating” group A β -hemolytic *Streptococci* and can be present along with *Staphylococcus aureus*.
- Type III – monomicrobial infections with gram negative bacteria. Its prevalence though is lower but associated with a high degree of mortality.
- Type IV – includes fungal infections

and is usually present in burn patients, post trauma or highly immunocompromised patients (1).

Pathophysiologically, NSTIs involve entrance of infectious organism(s) through epidermal breach (for example, due to trauma) causing soft tissue infections. Once the infectious organism(s) reach soft tissue, it releases exotoxins (M - proteins) which act as an antigen and have super antigenic activity (2). These endotoxins bind to the Vb domain of T-cell receptor and stimulate them resulting in inflammatory response of larger magnitude due to the release of proinflammatory cytokines interleukin (IL)-1, IL-6 and Tumor Necrosis Factor (TNF) - α . These inflammatory responses cause sepsis and shock and inhibit the adaptive immune responses of the host. Inside the infected soft tissue, there is generation of low tension, low pH environment and low redox potential which cause activation of Phospholipase C (PLC). Activated PLC boosts the formation of platelet-neutrophil complexes thus resulting in widespread thrombosis in blood vessels. This limits the blood supply to soft tissues causing their necrosis and preventing the host immune system to control infections. Furthermore, different types of endotoxin produce different clinical and/or biochemical outcomes. For example, Streptolysin O and S cause cell damage and degranulation of neutrophils resulting in the release of lysosomal enzymes which cause cell death. Streptokinase digests fibrin and lyses clots causing rapid spread of bacteria. Streptodornase degrades viscous DNA in necrotic tissues that along with hyaluronidase destroys cell structure and reduces viscosity of pus to cause characteristic "dish water pus" (2,3).

NSTIs affect superficial fascia leaving the skin characteristically unremarkable in initial phases of the disease. As bacteria/other microbes proliferate and toxins are released, localized necrosis ensures involvement of deep fascia and localized soft tissue. Based on the further spread of the disease, NSTIs have been classified into various stages (4,5):

- Stage 1 – The patient has erythema,

tenderness beyond erythema, swelling and local rise of temperature. In this stage, it is hard to distinguish the disease from cellulitis and erysipelas except disproportionate pain and the presence of systemic signs that would be absent in less severe infections.

- Stage 2 – This stage begins with the onset of critical skin ischemia. The patient has skin bullae, blisters and skin fluctuation. Blisters are formed due to ischemia induced necrosis.
- Stage 3 – As the disease evolves, there is frank necrosis of tissues. This leads to the presence of hemorrhagic bullae, skin anesthesia, necrotic patches and frank gangrene.

As the deeper layers of tissue including the fascia are involved, the disease spreads widely in the subcutaneous plane with the skin being involved much later. Often this may cause the disease to be overlooked in the initial phases or be confused with less aggressive infections such as cellulitis. Studies have proven that the time between presentation and surgical debridement directly affects the mortality of patient. For example, delay in surgical debridement by 12 hours can increase the mortality risk by 32%. Therefore, early diagnosis and early debridement plays an important role in saving the life and limb (6).

In the present work, we describe a step-by-step approach for the early management of NSTIs in North Indian population. To the best of our knowledge, no study has reported on the microbial profiling of necrotizing soft tissue based on the successful early management of these infections so as to minimize the mortality risk in North Indian population. To date, two studies (7,8) have been conducted in India. . While both studies provided microbial profiling of necrotizing tissues neither provided any recommendation for the early management of NSTIs. Hence, in the present work, we aim to propose an early diagnostic and management protocol for the successful management of NSTIs under clinical settings with the help of microbial profiling of necrotizing soft tissue.

Materials and Methods

A prospective study was conducted over a period of 1 year and 9 months (December 2017 to September, 2019) in a single tertiary center (Dayanand Medical College and Hospital, Ludhiana, Punjab, India) with 115 patients suffering from NSTIs. All the patients over 18 years of age with NSTIs requiring treatment in the form of surgical debridement were included in the study. Exclusion criteria were patients under 18 years of age or unwilling to undergo the treatment. Relevant information was collected from patients with the help of predesigned performa. The protocol of the study was approved by Baba Farid University of Health Sciences, Faridkot, Punjab (protocol no.: BFUHS/2K18p-TH/15706).

Treatment Protocol for the Early Management of NSTIs

The step-by-step protocol mentioned below was followed after the admission of the patient to the hospital:

1. Demographical data was collected (age and sex).
2. Determination of symptomatic features and risk factors: the symptomatic features included were pain, swelling, fever, oliguria and trauma. Associated risk factors with NSTIs include diabetes, alcoholism, chronic kidney disease, chronic liver disease, history of previous surgery, Body Mass Index (BMI) >30 or <18, hepatitis C, hypertension, burns, snake bite, immunosuppression, hypothyroidism, malignancy, child birth and others (including chicken pox, chronic obstructive pulmonary disease, old age (>75), paraplegia, pyelonephritis, steroid usage and ulcerative colitis).
3. Observation of clinical features for the diagnosis of NSTIs: included general, site specific and cutaneous features. Cutaneous signs were used as a primary parameter for the diagnosis of NSTIs severity.
4. Hematological analysis: to check the

presence of systemic infection, if any.

5. Treatment:

- (a) Initial resuscitation – All patients were treated with broad spectrum antibiotics, either penicillin or third generation cephalosporin with metronidazole or clindamycin. Intravenous (IV) fluids were started. If the patient had severe hypotension, inotropic support and invasive monitoring was initiated. Patients with poor GCS score or impending respiratory failure were put on ventilatory support.
 - (b) Surgical debridement – Depending on upon the clinical condition of the patient, surgical debridement was done as and when required.
 - (c) Limb amputation or diversion stoma – was done as per the condition of the patient or severity of NSTIs. Colostomy (usually sigmoid loop colostomy) or orchidectomy (usually in case of Fournier's gangrene) were used for perineal NSTIs.
6. Microbial profiling: Samples for microbial profiling could be withdrawn at different stages i.e., pre-operatively, intra-operatively and post-operatively. Samples collected could be infected tissue, pus or wound swabs of the infected area. They were then sent in sterile containers to the microbiology department for culture and sensitivity testing. Aerobic samples were first grown on MacConkey's agar and blood agar. Gram staining was done once growth was observed. For gram negative growths, MacConkey's agar media was used whereas for gram positive growths, blood agar media was used for further analysis. Growth was further continued manually with drug sensitivity checked by Kirby-Bauer method or automated Vitek 2. Similarly, anaerobic cultures were first passed through Robertson's meat broth medium and then grown on blood agar media. Fungal cultures were grown on Sabouraud's dextrose agar.

Based on the origin of the infection (polymicrobial /monomicrobial/fungal) and drug sensitivity testing, suitable pharmacological treatment was initiated.

In addition, the need for intensive care and the association between various risk factors and risk of mortality in patients suffering from NSTIs was also determined.

Results and Discussion

In the present study, the enrolled patients had ages between 18 – 86 years with a mean age of 52.3 years. About 46.1% patients (n=53) belonged to the age group of 51 – 70 years. NSTIs were found to be more prevalent among males (80.9%) as compared to females (19.1%). Most prominent symptomatic features were pain (97.4%), swelling (94.8%) and fever (64.3%) followed by oliguria and trauma. The most prominent risk factors for NSTIs were found to be diabetes (60%), alcoholism (11.3%) and chronic diseases of liver and kidneys (8.7% each).

General clinical features showed that most of the patients had tachycardia (56 – 138 bpm, mean 92 bpm). Tachypnea was uncommon and the mean respiratory rate was 20/minute. Only 3.5% (n=4) patients were febrile on presentation, 7.8% patients (n=9) had altered sensorium whereas 21.7% patients (n=25) presented with hypotension and shock (systolic blood pressure <90). All these clinical features pointed to the fact that most of the patients sought medical assistance in early stages of their disease prior to the onset of sepsis and septic shock. The most common areas of the body developing NSTIs were extremities followed by Fournier's gangrene or necrotizing fasciitis of the perineum. About 61.7% cases (n=71) were of lower limb infections; 14.8% cases (n=17) had the perineum involved; 11.3% cases (n=13) had upper limb infections while the rest of the cases were of abdominal wall and gluteal region.

While diagnosing the severity of NSTIs based on cutaneous signs, we found that most of the patients had presentation similar to cellulitis with tenderness (99.1% cases), edema (96.5% cases), local rise in temperature (93.9% cases), skin discoloration or necrotic patch (84.3% cases), dish wash pus (81.7%) and erythema (83.5% cases). Other signs such as blis-

ters, bullae and crepitus were rare (<12% cases). Another significant observation found was that only 2.6% of cases in the present work had blood infections while previous studies (7-11) reported its prevalence from 12.5 – 20.5%. It means that patients had Stage I NSTIs. The microbial profile of blood samples taken was also different from previous works (7-11). Collected blood samples mainly had *Acinetobacter baumannii* as main pathological agent while in previous studies, *Streptococcus*, *Vibrio* and *Staphylococcus* were found to be the major pathological organisms. All these observations along with the observed clinical features (Stage I) suggested that the enrolled patients sought medical attention in the early stages of the disease and we were able to detect the disease well in time.

After initial resuscitation, 111 patients underwent surgical debridement while the rest of the 4 patients died before the initiation of surgical interventions. Of these, 70 patients (60.9% cases) underwent single surgical debridement, 37 patients (32.2% cases) underwent debridement twice, 3 patients (2.6% cases) required it thrice while only 1 patient



Figure 1. A case of NSTIs of thigh in a 38 year old female with bronchial asthma on steroids - (A) at the time of clinical presentation, (B) post debridement (4 times)

(0.9% cases) required debridement four times (Fig. 1). In patients having NSTIs in extremities, 7 patients (6.1% cases) required single limb amputation while 1 patient (0.9% cases) required multiple limb amputation. In the patients with amputation, the main risk factor was found to be diabetes (62.5% cases, n= 5) and 87.5% patients (n=7) had necrosis of muscles on operative findings. One patient with Fournier's gangrene required orchidectomy. In NSTIs of perianal region and Fournier's gangrene, 6 patients (5.2% cases) required diverting stoma usually in the form of sigmoid loop colostomy. In our operative observations we found that the majority of the patients had involvement of subcutaneous tissue (99.1% cases). Among them, 9 patients had superficial disease not involving the underlying fascia while 103 patients (89.6% cases) had fascial involvement and 77 patients (67% cases) had myonecrosis.

For microbial profiling, we collected 60 samples of infected tissue, 26 samples of pus and 29 samples of wound swabs. Blood culture in most of the patients (97.4% cases) did not have any growth or bacteremia. Out of three patients who showed growth, two (1.7% cases) had *Acinetobacter* and one (0.9%) showed the growth of Methicillin Resistant *Staphylococcus aureus*. This confirmed that most of the patients did not have blood infection at the time of admission and were in an initial phase of the infection (Stage 1).

Out 115 patients, samples of 103 patients showed mono microbial (89.6% cases) growth while 12 patients had polymicrobial growth (10.4% cases). Two patients (1.7% cases) also showed fungal hyphae on smear. The distribution of pathogenic organisms in polymicrobial and mono microbial infections is presented in Table 1.

It is evident from Table 1 that monomicrobial infections were more prominent than polymicrobial infections and among monomicrobial infections, gram negative infections were more prominent in the selected population (Type III NSTIs). These types of infections are more lethal and are associated with high risk of mortality. The most preva-

Table 1. Distribution of pathogenic organisms in NSTIs in 115 patients

Organism	Percentage
Polymicrobial infections (n=12)	
<i>Gram negative bacteria</i>	
<i>Acinetobacter baumannii</i>	66.6
<i>Escherichia coli</i>	25
<i>Pseudomonas aeruginosa</i>	41.6
<i>Citrobacter koseri</i>	8.3
<i>Klebsiella pneumoniae</i>	41.6
<i>Providentia stuartii</i>	8.3
<i>Morganella morganii</i>	8.3
<i>Gram positive bacteria</i>	
Methicillin Sensitive <i>Staphylococcus aureus</i>	8.3
<i>Enterococcus faecium</i>	16.6
<i>Fungus</i>	
Fungal hyphae	8.3
Monomicrobial infections (n=103)	
<i>Gram positive bacteria</i>	
Methicillin Resistant <i>Staphylococcus aureus</i>	0.9
Methicillin Sensitive <i>Staphylococcus aureus</i>	9.6
<i>Staphylococcus hemolyticus</i>	0.9
<i>Enterococcus faecium</i>	0.9
<i>Streptococcus</i>	0.9
<i>Gram negative bacteria</i>	
<i>Acinetobacter baumannii</i>	19.1
<i>Escherichia coli</i>	40.0
<i>Enterobacter cloacae</i>	3.5
<i>Citrobacter koseri</i>	0.9
<i>Klebsiella pneumoniae</i>	11.3
<i>Providentia stuartii</i>	1.7
<i>Pseudomonas aeruginosa</i>	6.1
<i>Clostridium perfringens</i>	0.9
<i>Stenotrophomonas maltophilia</i>	0.9
<i>Morganella morganii</i>	0.9
<i>Others</i>	
Fungal hyphae	1.7

lent pathogen found in the collected samples was *Escherichia coli* (in 40% of the samples). This observation was contradictory to previous reports which showed the prevalence of polymicrobial infections more as compared to the monomicrobial infections. This means that the type of infections can also vary based on geographical location. This is an important observation made in our study. Further, since we were able to detect the condition in an initial stage of type III NSCIs, we were able to reduce the mortality substantially (10.4%) which was quite low as compared to the previous reports (> 20%) (7-11). The major portion of the test population was diabetic, therefore separate analysis was done for 69 diabetic subjects. It was found that they also

had high prevalence of monomicrobial gram negative infection in which the percentage of *Acinetobacter*, *E. coli*, *Klebsiella* and *pseudomonas* was 21.7%, 42.02%, 14.4% and 5.7%, respectively. Depending upon the culture and sensitivity reports, Amikacin, Meropenem, Colistin, Imipenem, Ciprofloxacin, Tigecycline, Gentamycin, Minocycline, Cefepime, Levofloxacin, Doripenam, Rifampicin, Teicoplanin and Vancomycin were found to be the most effective drugs against NSTIs.

All patients with NSTIs were also classified according to their need for intensive care at any point of disease duration. Intensive care is an indicator of increased morbidity. It was found that 45.2% patients (n=52) required intensive care in the present study. The demographic data, symptomatic and clinical features, risk factors, operative findings, biochemical parameters and growth of cultures were compared to the risk of need of intensive care by univariate regression analysis. It was found that male gender ($p=0.016$), decreased urine output ($p<0.001$), trauma ($p=0.04$), fever ($p=0.02$), diabetes ($p=0.02$), chronic kidney disease ($p=0.04$), amputation ($p=0.02$) and muscle involvement ($p=0.005$) were associated with the need for intensive care to a statistically significant level.

NSTIs if not treated promptly and adequately with surgical debridement can lead to high rate of mortality among patients. Due to our early management of NSTIs, we observed a mortality rate of only 10.4% (12 cases out of 115 cases). Based on univariate regression analysis we found significant association between fever ($p=0.05$), BMI ($p=0.02$), blisters ($p=0.005$), amputation ($p=0.003$) and *Acinetobacter* infection ($p=0.05$) with a high risk of mortality.

Conclusion

Necrotizing soft tissue infection is a rapidly spreading infectious disease that can prove fatal if not treated adequately and urgently. Depending upon the findings observed in our study, we conclude that NSTIs are more prevalent among males having diabetes in

North Indian population with monomicrobial gram negative infections (Type III) being the most common and *Escherichia coli* being the most prevalent pathogenic organism. The step-by-step approach followed in the present study helped in the early detection and management of patients with NSTIs effectively resulting in a significant decrease in mortality (10.4%) and limb loss.

Conflict of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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Data Availability

Raw data of present study is available with corresponding author and can be obtained after request.

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