ORIGINAL ARTICLE

Impaction of the polylactic membrane or hydrofiber with silver dressings on the interleukin-6, tumor necrosis factor-α, transforming growth factor-β_3 levels in the blood and tissues of pediatric patients with burns

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ABSTRACT

BACKGROUND: We aimed to evaluate the effects of two different burn dressings, hydrofiber with a silver (HFAg) and polylactic membrane (PLM), on altering the levels of important biomarkers Interleukin-6 (IL-6), Tumor necrosis factor-α (TNF-α), Transforming growth factor-β_3 (TGF-β_3) in blood and burnt tissue in children with second-degree burns.

METHODS: Children between the ages of one to 16 years, with 25–50% second-degree partial-thickness burns of the total body surface area were included in this study. Patients in the PLM group were dressed with PLM in a typical way according to the manual. The HFAg group was dressed with HFAg and a sterile cover. During and at the end of the 21-day treatment, blood and skin tissue samples were taken from the two burn and control groups. IL-6, TNF-α, and TGF-β β_3 levels were evaluated in blood and tissue samples from all groups, and the results were analyzed statistically.

RESULTS: In the PLM group, IL-6 and TNF-α levels decreased early days in both serum and tissue samples to reach normal ranges compared with the HFAg group. In the PLM group, TGF-β_3 levels were elevated than in other groups for two weeks.

CONCLUSION: In this study, we found that PLM controls inflammation earlier in both systemic and burn tissue. We also found that PLM increased the level of TGF-β_3, which may be associated with the prevention of the development of hypertrophic scar in the burn wound, in the blood and burn tissue during this study.

Keywords: Burns; children; dressing; IL-6; hydrofiber with silver; polylactic membrane; TNF-α; TGF-β.

INTRODUCTION

Burn trauma is an essential cause of fatal and nonfatal injuries annually.[1] The American Burn Association (ABA) National Burn Repository 2017 provides an overview of 212.820 records treated in American burn centers within nine years and describes fire/flames and scald injuries as the etiology of 76% of the cases reported.[2] Then, the World Health Organiza-

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tion (WHO) estimates 265 000 casualties per year from fire without scalds, electric burns, and others.[3] Complications like shock, multi-organ failure, or cardiovascular failure are closely linked to morbidity and mortality,[4] even in a pediatric population where multi-organ failure is found in 25% of patients with a lower than 40% total body surface area (TBSA). The dominant age group inflicted is children younger than 10 years of age,[1] and the most common cause in the children's age group is scalding.

When more than 10% of TBSA is affected, severe and potentially fatal systemic pathophysiological effects are initiated if treated improperly.[5,6] These changes following burns arise from mediators released after trauma under the direct influence of the injury.[6] A cascade of cytokines, complement activation, and liberation of free radicals is initiated by the innate immune system with the physiological intention to clean the wounds and eliminate dead tissue. Early disbalance of inflammatory and anti-inflammatory cytokines in more extensive burns yields a systemic inflammatory reaction with a capillary leak, edema, organ failure, and hypermetabolism. TNF-α and IL-6 are components responsible for the proinflammatory triggering of this response after burning.[6] TGF-β is one of the most important cytokines influencing the healing process of burn wounds.[7] During the burn healing and treatment process, the levels of these biomarkers change both in the tissue and in the blood over time.[6]

A burn is a pathological condition with severe systemic and local skin damage effects.[5] After a severe burn, tissue damage due to ischemia-reperfusion and other components, such as activation of the immune system by alarm proteins (DAMPs) along with thermal trauma may cause systemic inflammation in the body.[6,8] Activation of NOD-like receptors (NLRs) and Toll-like receptors (TLRs) play a crucial role in the emergence of this systemic effect.[9] TLR and NLR produce endogenous factors that initiate inflammation. They are activated and expressed by leukocytes, detecting fragmented cell particles as molecules that under normal conditions are in intracellular localization but can be released from the cell following cellular degradation.[9] TLR is the primary mechanism that detects the extracellular presence of these molecules.[9] NLR is a secondary mechanism that ensures the integrity of the cell membrane and is activated by detecting particles outside the cell from the disintegration of a typical cell membrane.[9] As studies made the mechanism of the systemic response to inflammation more evident,[10–12] the "Two-Hit Hypothesis" fits this concept well.[9–12] In the case of cellular damage, fragmented cells may induce an inflammatory response. If prolonged, the damage caused by it may lead to a new inflammatory reaction.[9]

Activated TLR provides gene expression that produces proinflammatory cytokines, while activated NLR triggers inflammatory caspases, such as caspase-1.[9,13,14] When both mechanisms are activated, it releases cytokines, such as TNF-α and IL-6,[9,13,14] which play a crucial role in the emergence of systemic and local pathological events following burn events.[6,9,15] TNF-α is produced by macrophages that cause metabolic and systemic inflammatory effects that are released into the bloodstream within a few minutes after tissue destruction. It also has a predictive value for the development of septic complications in patients with burns.[6] IL-6 is produced by T cells and plays a vital role in early inflammation, and its concentration in patients with burns reflects the initial inflammatory response.[6]

At the beginning of the inflammatory process, TGF-β responds to active inflammation.[7] In cases of deficiency during wound healing in patients with burns, wound recovery fails; organ failure may develop, leading to death.[16] It is produced by keratinocytes, platelets, macrophages, and lymphocytes.[17] TGF-β provides matrix formation, re-epithelialization, and granulation tissue formation in wound tissue.[17] TGF-β_1, TGF-β_2 and TGF-β_3 are the main forms found in mammals.[17] Although TGF-β_1 and TGF-β_2 cause the development of hypertrophic scar formation when over-expressed, increased expression of TGF-β3 prevents the hypertrophic scar to develop.[17]

Hydrofiber with a silver (HFAg) and polylactic membrane (PLM) are two dressing materials used as covers for wounds in burn treatment.[18,19]

This study aimed to evaluate the influence of these burn dressings (HFAg and PLM) on altering biomarker levels (IL-6, TNF-α, and TGF-β3) in blood and burnt tissue in children with second-degree burns.

MATERIALS AND METHODS

The Ethics Committee of the Clinical Research Committee of İnönü University School of Medicine (2018/72) approved this study. We performed this prospective and randomized study as a single center in the Pediatric Burn Center in Turgut Özal Medical Center. Two independent evaluators with long clinical experience performed wound evaluations clinically (30 and 14 years, respectively). The pain was recorded using a visual analog scale (VAS) as procedural and resting pain. The bleeding was evaluated clinically and distinguished into three degrees (+, ++, +++). The effusion was described clinically and classified into three degrees (+, ++, +++).

Statistical Analysis

Normally distributed data were summarized by means and standard deviations. The Levene test was used to test the homogeneity of the group variances. When group variances were homogeneous in one-way analysis of variance, Tukey's and Tamhane's T2 post-hoc methods and the Welch test were used. Non-normal data were expressed as median, minimum, and maximum values. For these data, group comparisons

were performed using the Kruskal-Wallis test and Conover's pairwise comparison method. In all analyses, the significance level was set at 0.05.

Group Formations

All patients with partial-thickness burns at the age of 1–16 years were identified and asked to participate in this study. Informed consent was obtained from all eligible parents of participants. During the study period, 284 patients were eligible for inclusion; 108 patients were excluded from this study based on our exclusion criteria; 38 patients refused to participate in the survey; 95 patients were excluded due to some reasons (e.g., the emergence of sepsis, consent not feasible). A total of 65 children were included in this study. Of these, 22, 21, and 22 patients were in the PLM, HFAg, and control groups, respectively. The demographic information of patients in the two dressing and control groups is shown in Table 1. Individuals in the control group were created without systemic disease from girls with inguinal hernia patients and would be circumcised boys in elective conditions between the ages of 1 and 16.

Evaluation: All patients were evaluated clinically concerning the extent and depth of burns by two specialists experienced in burn treatment. Medical procedures were performed as well.

Inclusion Criteria: Patients between the ages of 1–16 years with scald, flame, and contact burns, with adequate resuscitation after injuries with the extent of TBSA not only 25% to 50% superficial partial-thickness but also 10% of those with deep partial-thickness wounds were deemed eligible for our study.

Exclusion Criteria: Patients with inhalation injury, respiratory disease, systemic disease, acute renal failure, and those who developed sepsis previously were excluded from this study. Patients undergoing surgery, recipients of erythrocyte suspension, experienced other trauma, or electrical or chemical burns were also excluded from this study.

Randomization for Group Formation

Randomization was performed by the sequence of patients consenting in the study in a 1:1 manner. This procedure was documented using a randomization protocol.

Treatment: All burned patients were administered the same essential standard burn treatment[20] regarding fluid treatment, daily calorie requirement, and oral nutrition of all patients, which was arranged by the same nutritionist. Patients in the PLM group were dressed with PLM in a typical way according to the manual. The HFAg group was dressed with HFAg and a sterile cover.

Dressing Protocols

After the primary survey during the first application, the burnt wound was properly washed with sterile gauze with serum saline and cleaned. If debridement was necessary, the surgical sponge was used. After, the wound was dried and covered with the appropriate wound dressing.[21] The order in which dressing was used was, respectively, one by one.

Dressing Types

The HFAg (AquacelAg®, Convatec, Princeton, NJ, USA) is a hydro-fiber, burn wound dressing that is a type of fleece material made up of sodium carboxymethyl cellulose fibers that switch to gel form when they are exposed to the wound

Table 1. Patients demographic

Variable	Group of PLM (n=22)	Group of HFAg (n=21)	Group of Control (n=22)	P
Age (years)	4.75±3.70	4.95±3.76	4.82±3.15	NS
Gender (male/female)	11/11	11/10	11/11	NS
Type of Burn (n. %)				NS
Scald	14	13		
Flame	7	7		
Contact	1	1		
Hernia or circumcision				
Hernia			Right Inguinal Hernia (n=11)	
Circumcision			Circumcision (n=11)	
TBSA burn (%)	30.85±4.42	31.15±4.32		NS
Deep burn (%)	6.7±1.23	6.6±1.20		NS
Length of intensive care unit (days)	2.55±0.72	2.65±0.62		NS
Length of hospital stay (days)	21.4±1.6	24.3±1.7		NS

PLM: Polylactic membrane; HFAg: Hydrofiber dressing with silver; NS: Nonspecific; TBSA: Total body surface area.

containing 1.2% silver in an ionic form.[18] The HFAg of the patients in this group (n=21) was changed on days 0, 7, and 14, and the dressing changes were repeated weekly until wound epithelialization was completed.

The PLM (Suprathel®, PMI GmbH, Denkendorf, Germany) is a synthetic copolymer and a burn wound dressing consisting of more than 70% DL-lactic acid and ε-caprolactone.[19] The dressing cover of the patients in the PLM group (n=22) was changed on days 0, 7, and 14, and the dressing changes were repeated weekly until wound epithelialization was completed.

Clinical Protocols

All patients were evaluated in terms of resting and procedural pain levels (not published in this study), macroscopic re-epithelialization, and progression of epithelialization at the edges of the wound, bleeding (results not part of this study), and decreased exudate (results not part of this study) during each dressing procedure. The burn wounds of all patients were visually evaluated by two experts who were experienced.

Clinical tests were performed to show the effects of how two different burn dressings changed the systemic and local effects of second-degree burns for 21 days. We investigated the immunohistochemical levels in burnt tissue and systemic levels in the blood of TNF-α, IL-6, and TGF-β_3, which play an essential role in the systemic and regional effects of the burn.

Blood Sample Collection from Patients and Control Groups

In the dressing groups on the 1st, 3rd, 7th, 10th, 14th, and 21st day after burns, blood samples were collected in biochemistry tubes. Blood samples were taken from the children in the control group at once on the 1st arrival at the hospital. Blood was taken from all the control group children on the same day. An average of 5–10 ml of blood was taken from children in the control group, between 09:00 and 10:00 in the morning. The control group serum parameters of this study were obtained from this blood. The serum was separated by centrifuging the blood samples at 1600 x g for seven min; samples were stored at -80°C and kept until analysis.

Biochemical Analysis

The levels of IL-6, TNF-α and TGF-β3 were analyzed by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Bioassay Technology Laboratory, Shanghai, China). We also used an ELISA microplate reader (BioTek Synergy H1, BioTek Instruments) and the data analysis program (Gen5, BioTek Instruments).

Tissue Samples

Biopsies were taken from burned skin on the first, 7th, 14th, and 21st days. A skin biopsy, 0.2 mm in size, was performed under local anesthesia with 2% lidocaine (Priloc® VEM Drug Industry and Trade Co, Ankara, Turkey), preferably from the right upper extremity of the burnt skin and of the forearm due to the sun-protected interior. If the right upper extremity was not suitable, the biopsy was taken from the sun-protected area of the top left extremity. If both upper extremities were not appropriate, the biopsy was performed after local anesthesia from the lower extremity or the sun-protected burnt area of the body.

In boys in the control group, after circumcision, tissue samples were taken from the prepisium skin. In girls in the control group, during inguinal hernia surgery, the surgical site was excized the skin of 0.2 mm in size.

The tissues in the biopsy sample were subjected to routine tissue examination and stored in paraffin blocks, as in our previous study.[22] These specimens were immunohistochemically stained for TNF-α, IL-6, and TGF-β β_3 expression. Immunohistopathologic evaluation was performed in a blinded manner by two pathologists.

Immunohistochemical (IHC) Methods and Histopathological Evaluation

In this study, skin biopsy samples were first stained with Hematoxylin eosin H&E and then prepared as described in our previous study.[22] After a five minute exposure to peroxidase extinguishing solution, blocks were treated using EDTA (Ethylenediamine tetraacetic acid) before further processing.

Slides were incubated overnight in 200 µL of anti-IL-6 antibody (Sc-28343 primary antibody solution, Santa Cruz Biotech Inc, Dallas, TX, USA), anti-TGF-β_3 antibody (Sc-166861 Santa Cruz Biotech Inc, Dallas, TX, USA), and anti-TNF–α (Sc-52746, Santa Cruz Biotech Inc., Dallas, TX, USA). Slides were then incubated with secondary antibodies. The slides were then prepared for evaluation. Examples of immunohistochemical staining are shown in Figure 1a-c.

Our cell counts were performed with an immersion magnification using a grid in the epidermis. We utilized 525-micron² areas in 2-micron sections. In the census with the multipoint tool, cells with TNF-α, IL-6, and TGF-β antibody and intense cytoplasmic staining were then counted in the cytoplasm of all cells.[23–25] In the epidermis, the cell cytoplasm was evaluated from the stratum basale to the stratum corneum. The counting areas were based on photos obtained from 40X magnification, depending on the size of the texture.

Due to different epidermis thicknesses in different areas, at least three to six field photographs were used from each tissue.[23–25] After counting up to 1,000 cells in total, the ratio of total squamous cell cytoplasm stained and unstained with TNF-α, IL-6, and TGF-β antibody were calculated in each census. Statistical testing was performed to determine significant differences. However, at present, many available pro-

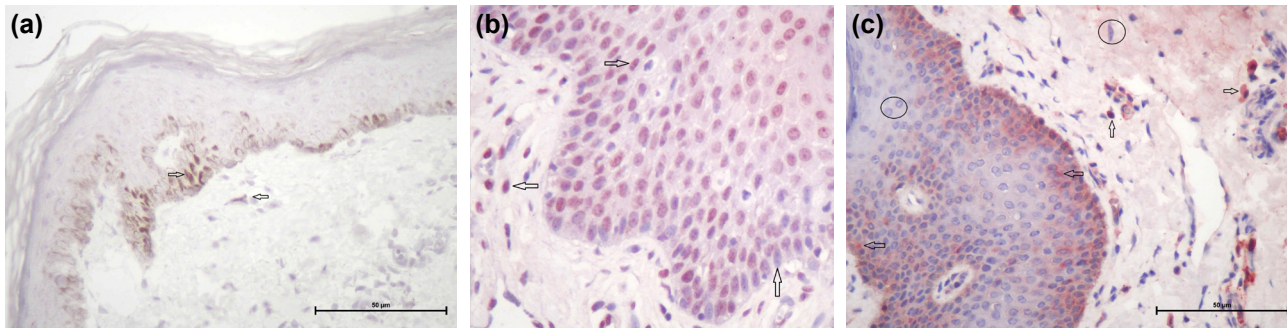

Figure 1. (a) IL-6 expression from the burnt tissue of a patient from the HFAg group. The nuclear expression with the phosphorylated STAT3 activation of IL-6 commonly in basal keratinocytes is shown to the right. The left arrow indicates the nuclear expression with the activation of STAT3 with phosphorylated IL-6 in fibroblasts. The upper arrow indicates the loss of the nuclear expression in keratinocytes with the activation of the phosphorylated STAT3 of IL-l6. **(b)** TGF-β_3 expression from the burnt skin of a patient from the PLM group. The right arrow shows the widespread TGF-β_3 cytoplasmic expression in basal keratinocytes. **(c)** TNF-α expression from the burnt skin of a patient from the HFAg group. The left arrow shows the TNF-α cytoplasmic expression in basal and superficial keratinocytes. The right arrow shows cytoplasmic expression in macrophages. It shows cytoplasmic expression in the upward arrow lymphocyte. Some oval keratinocytes and fibroblasts did not show any expression.

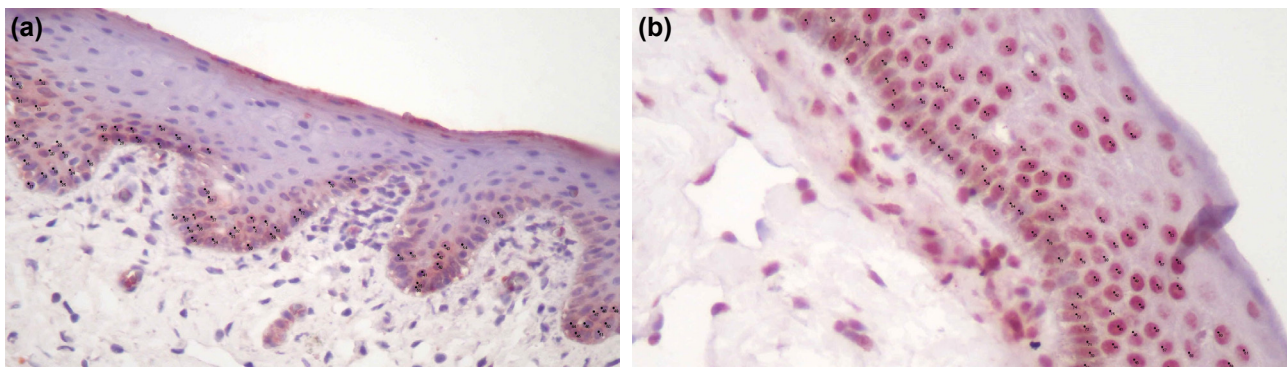

Figure 2. TNF-α expression from burnt skin. Images were analyzed using Image-J and are at magnification of 10X **(a)** and 40X **(b)**, respectively.

grams provide data on microscopic images through automated or manual counting. Our census was conducted using the open-source "Image J" program (Fig. 2a, b).[23–25]

RESULTS

When the demographic data of the three groups were analyzed statistically, there was no significant difference (Table 1).

The serum analysis of TNF-α, IL-6, and TGF-β β_3 levels in all groups during the 21-day study period is shown in Tables 2–4. Figure 3a-c reflects the change in serum levels of groups over time.

Serum IL-6 levels were significantly higher on the first day in both the PLM and HFAg groups compared with that of the control group. In the PLM group, the IL-6 level was significant-

Table 2. Results of statistical analysis of serum IL-6 (ngml) levels between groups

Group	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21
Control (n=22)	4.53 (1.29–9.24) ^{x}	4.53 (1.29–9.24) ^{x}	4.53 (1.29–9.24) ^{x}	4.53 (1.29–9.24) ^{x}	4.53 (1.29–9.24) ^{x}	4.53 (1.29–9.24) ^{x}
HFAg (n=21)	126.28 (19.53–211.47) ^{y}	236.28 (155.28–342.83) ^{y}	326.96 (215.13–427.63) ^{y}	225.13 (155.28–427.63) ^{y}	114.5 (35.03–188.01) ^{y}	6.44 (2.6–14.24) ^{y}
PLM (n=22)	121.73 (101.99–211.83) ^{y}	214.42 (144.82–255.03) ^{z}	216.62 (113.98–419.11) ^{z}	102.96 (10.31–165.05) ^{z}	4.79 (3.55–15.45) ^{z}	4.43 (1.35–7.77) ^{x}
p	<0.001	<0.001	<0.001	<0.001	<0.001	0.002

*The difference between the groups with different superscript letters was statistically significant. PLM: Polylactic membrane; HFAg: Hydrofiber dressing with silver.

Table 3. Results of statistical analysis of serum TNF-α (ngml) levels between groups

Group	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21
Control (n=22)	15.46 (2.63–30.56) [x]	15.46 (2.63–30.56) [x]	15.46 (2.63–30.56) [x]	15.46 (2.63–30.56) [x]	15.46 (2.63–30.56) [x]	15.46 (2.63–30.56)
HFAg (n=21)	812.16 (511.87–885.95) [y]	550.52 (420.85–821.62) [y]	335.51 (211.63–553.75) [y]	234.43 (135.51–380.78) [y]	141.4 (95.51–191.63) [y]	15.41 (5.63–47.65)
PLM (n=22)	811.5 (511.87–885.95) [y]	595.55 (495.99–881.62) [y]	344.97 (276.73–563.75) [y]	132.92 (85.51–183.12) [z]	15.46 (5.63–42.85) [x]	16.75 (5.63–26.68)
p	<0.001	<0.001	<0.001	<0.001	<0.001	0.937

*The difference between the groups with different superscript letters was statistically significant. PLM: Polylactic membrane; HFAg: Hydrofiber dressing with silver.

Table 4. Results of statistical analysis of serum TGF-β_3 (ngml) levels between groups

Group	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21
Control (n=22)	37.26 (23.67–74.56) [x]	37.26 (23.67–74.56) [x]	37.26 (23.67–74.56) [x]	37.26 (23.67–74.56) [x]	37.26 (23.67–74.56) [x]	37.26 (23.67–74.56) [x]
HFAg (n=21)	198.74 (66.1–1006.86) [y]	325.24 (194.91–565.24) [y]	333.04 (234.24–563.23) [y]	354.24 (233.8–1404.5) [y]	333.48 (223.42–854.42) [y]	153.86 (73.74–364.5) [y]
PLM (n=22)	235.86 (114.74–756.1) [y]	535.24 (255.1–756.98) [z]	1434.43 (753.13–2895) [z]	1438.49 (755.02–2535.29) [z]	803.83 (356.74–1424.5) [z]	455.92 (224.12–1023.86) [z]
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*The difference between the groups with different superscript letters was statistically significant. PLM: Polylactic membrane; HFAg: Hydrofiber dressing with silver.

ly lower than that in the HFAg group from the 3rd day of this study. The IL-6 concentration in the PLM group started to decrease from the 7th day onward and dropped to the same level as the control group on the 14th day. The IL-6 concentration in the HFAg group peaked on the 7th day and then began to decrease. However, even on the 21st day, it was still significantly higher than in the control group (Table 2 and Fig. 3a).

Serum TNF-α level was very high in both the PLM and HFAg groups on the first day. There was no difference between the groups. Serum TNF-α levels decreased significantly in the PLM group starting on day 10 compared to the HFAg group and reduced to the same level as the control group on the 14th day. In the HFAg group, the serum TNF-α level reached the same level as the control group on the 21st day (Table 3, Fig. 3b).

Serum TGF-β_3 levels in both the PLM and the HFAg groups were higher on the first day compared with that of the control group. The PLM group showed the highest serum TGF-β_3 level after the 3rd day, compared to the other groups ($p<0.001$). Although it dropped, the serum TGF-β_3 level in the PLM group was higher than that in the two other groups, even on the 21st day. In the HFAg group, the serum TGF-β_3 level was higher on the first and in the following days compared with that of the control group, but lower than that of the PLM group (Table 4, Fig. 3c).

The findings in skin tissue samples taken during the entire study of the HFAg, PLM, and control groups are shown in Tables 5–7 and Figure 4a-c.

Both in the PLM and HFAg groups, the tissue expression of

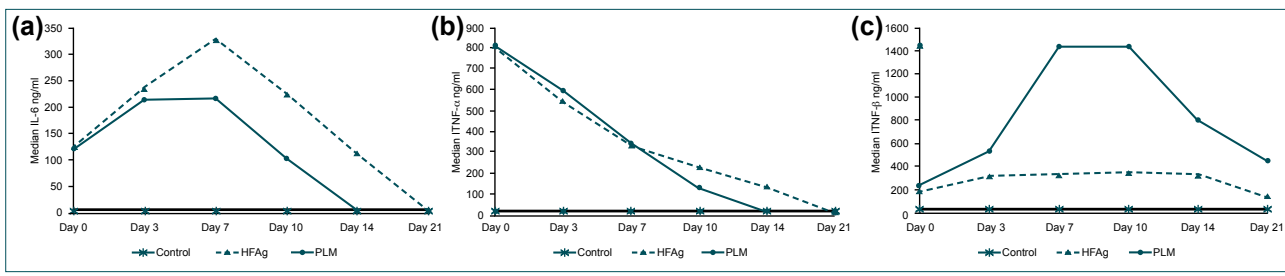

Figure 3. Change over time in serum **(a)** IL-6, **(b)** TNF-α and **(c)** TGF-β_3 levels across the three groups.

Table 5. Statistical analysis of the skin TNF-α expression evaluation of the groups

Ratio of TNF-α expression %	Control (n=22)	HFAg (n=21)	PLM (n=22)	p
Day 1	2.9±1.24[a]	49.74±15.45[b]	50.39±13.63[b]	<0.001
Day 7	2.9±1.24[a]	33.42±8.71[b]	14.01±7.77[c]	<0.001
Day 14	2.9±1.24[a]	9.27±6.91[b]	2.93±1.32[a]	0.001
Day 21	2.9±1.24	2.94±1.27	2.91±1.16	0.995

*The difference between the groups with different superscript letters was statistically significant. PLM: Polylactic membrane; HFAg: Hydrofiber dressing with silver.

Table 6. Statistical analysis of the skin IL-6 expression evaluation of the groups

Ratio of IL-6 expression %	Control (n=22)	HFAg (n=21)	PLM (n=22)	p
Day 1	2.35±1.13[a]	55.04±15.94[b]	54.86±12.88[b]	<0.001
Day 7	2.35±1.13[a]	34.52±9.61[b]	11.61±6.1[c]	<0.001
Day 14	2.35±1.13[a]	10.72±7.63[b]	2.67±1.23[a]	<0.001
Day 21	2.35±1.13	2.46±1.04	2.48±1.18	0.921

*The difference between the groups with different superscript letters was statistically significant. PLM: Polylactic membrane; HFAg: Hydrofiber dressing with silver.

IL-6 was higher than in the control group for samples taken from the 1st and the 7th day after the burn. In the PLM group, burnt skin IL-6 expression was lower than in the HFAg group on day 7 but higher than that of the control group. In the PLM group, IL-6 expression dropped to the same level as in the control group on the 14th day. In the HFAg group, IL-6 expression decreased to the same level as that of the control group on the 21st day (Table 5 and Fig. 4a).

Tissue TNF-α expression, both in the PLM and HFAg groups, was significantly higher than that of the control group in samples taken from day 1 to 7 after burns. In the PLM group, burnt skin TNF-α expression was lower than that of the HFAg group on the 7th day but higher than that of the control group. In the PLM group, TNF-α expression decreased to the same level as the control group on the 14th day. HFAg group TNF-α expression decreased to the same level as the control group on the 21st day (Table 6 and Fig. 4b).

Skin tissue TGF-β_3 expression was not different from the control group in all groups on the first day. HFAg, levels were higher in the burnt skin group than in the control group on days 7 and 14. No differences were found on the 21st day.

In the PLM group, burnt skin TGF-β_3 expression was significantly higher than in the other two groups on days 7, 14, and 21. The TGF-β_3 expression peaked on day 14, and then began to decrease, returning to a still-elevated level in the third week with a 40% drop within the last week (Table 7 and Fig. 4c).

Clinical Evaluation

In this study, two different dressings were applied to burnt patients. The influence on IL-6, TGF-β_3, and TNF-α levels of these different methods was examined during the 21 days treatment. The essential proinflammatory biomarkers Il-6 and TNF-α, when compared with the control group, were significantly higher in the first seven days in both study groups. In the PLM group, IL-6 and TNF-α levels began to decrease earlier in both serum and tissue and decreased to normal levels earlier than in the other groups. Therefore, the proinflammatory effect of the burn injury was mitigated using PLM treatment.

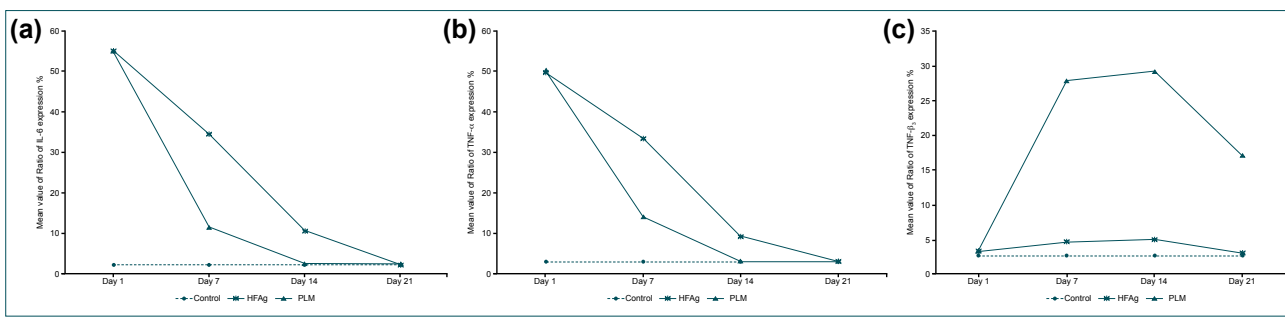

Figure 4. Change over time in skin tissue **(a)** IL-6, **(b)** TNF-α and **(c)** TGF-β_3 expression across the three groups.

Table 7. Statistical analysis of the skin TGF-β_3 expression evaluation of the groups

Ratio of TGF-β_3 expression %	Control (n=22)	HFAg (n=21)	PLM (n=22)	p
Day 1	2.81±1.33	3.48±1.67	3.47±1.51	0.252
Day 7	2.81±1.33[a]	4.77±2.14[b]	27.88±15.99[c]	<0.001
Day 14	2.81±1.33[a]	5.11±2.06[b]	29.2±16.06[c]	<0.001
Day 21	2.81±1.33[a]	3.16±1.49[a]	17.18±10.05[b]	<0.001

*The difference between the groups with different superscript letters was statistically significant. PLM: Polylactic membrane; HFAg: Hydrofiber dressing with silver.

TGF-β_3 is a factor that regulates and positively affects wound healing. It was higher than in the other group for two weeks and then dropped in the PLM group. After 21 days of treatment, the level was more elevated compared with that of the other groups. No data were collected after the third week to see whether TGF-β_3 remained at an elevated level.

In this study, IL-6 and TNF-α, which are systemic inflammation indicators during injuries in the PLM group, were initially very high, similar to other groups. However, in contrast to the others, it decreased in a short period and reached the same levels as the control group.

DISCUSSION

Many experimental and clinical studies have been conducted comparing different burn dressings concerning tissue and systemic impact.[21,22,26–28] In our opinion, the molecular basis of pathological events that arise from burns should be well understood to interpret the emerging results. For this reason, we assessed biomarkers with an essential role in the development of pathological events in burn and wound healing. We believe that a better understanding of the purpose and outcome of this study may contribute significantly to the knowledge about treatment modalities and improve the successful treatment of patients with burns.

Karimi et al.[26] evaluated the histopathological changes in second-degree skin burns treated with two different silver dressings (AgiCoat® and Acticoat®) in the liver, lung, kidney, and brain tissues. Although wound healing was faster in the Acticoat group, they identified toxic effects in the liver in both groups. This unwanted effect was more prominent in the AgiCoat group.

Karlsson et al.[27] evaluated the efficacy of silver foam compared to porcine xenograft in partial-thickness scald burn in a prospective randomized clinical study. Parameters were the time for wound healing and pain. They observed that the silver-foam group was more successful.

Denzinger et al.[28] explored the interaction of different burn wound dressings on blood. Ex vivo studies found that Xenograft, Jelonet®, and Matriderm® had a hemostyptic effect and that EZ-Derm® and Burntec® were able to activate the complement system.

Some studies have evaluated the efficacy of silver-containing dressing materials in wound healing of burns and autograft donor regions.[18,21,29–31] These studies suggested that HFAg had a cytotoxic effect, negatively affecting wound healing despite its antibacterial efficacy due to the silver content.[21,29–36] Our investigations related to the impaction of different burn dressings on telomere kinetics revealed that HFAg reduces the number of keratinocytes in burn tissue.[22] The antibacterial efficacy of silver was attributed to the inhibition of matrix metalloproteinase in a study[32] and another study suggested that silver increased apoptosis in wound bed cells.[33]

In our previous study, we found that wound healing was longer in children with partial-thickness burns treated with HFAg than with PLM. Inflammatory cytokines (IL-6 and TNF-α) were elevated for extended periods and returned to normal later in patients with burns treated with HFAg. Our findings on proinflammatory cytokines in this study were consistent with our previous evaluation of oxidative stress.[21]

The lactate released by degradation from PLM components is claimed to have an active defensive role against free oxygen radicals[37–39] due to the ability to scavenge free radical particles in the extracellular compound. Our findings support this alleged effect.[37] The scavenging of free radicals released in a burn might attenuate the inflammatory response.

Il-6 and TNF-α, two proinflammatory biomarkers that increase the severity and adverse effects of inflammation, were lower in burnt tissue and blood in the group of patients treated with PLM, compared with that of the HFAg group. This effect might also be due to the free radical scavenging ability of lactate from PLM.

Lu et al.[40] investigated the *in vitro* effects of TGF-β β_3 on wound healing. They compared normal skin fibroblasts and hypertrophic scar fibroblasts in culture. When the same dose of TGF-β_3 to both groups, hypertrophic scar fibroblasts produced more type III procollagen, and the ratio of+ class I to 3 procollagen was decreased in normal fibroblasts. They hypothesized the influence of TGF-β_3 "to be beneficial and accelerate wound healing and inhibit or prevent scar formation.[40]

The formation of hypertrophic scar tissue during and after wound healing in burn wounds is not fully understood, but type III collagen is produced more in scar tissue.[7]

We found that TGF-β_3 in the PLM group was significantly higher within the first two weeks and decreased rapidly from day 10 to low levels after the third week. The sharp rise and fast drop of serum TGF-β_3 after day 10 in the PLM group and the reduced inflammatory response might avoid the predisposition for hypertrophic scarring due to the increased formation of myofibroblasts.[41]

In our study, all patients in the treatment groups underwent routine full blood tests every week during the study. All blood results were normal after the first week.

Conclusion

Inflammation is one of the main components of burn damage. Different burn dressings influence the amount of inflammatory response present. The PLM controlled inflammation earlier in both systemic and burnt tissues. Also, PLM indirectly improved wound healing by influencing the inflammation response. We believe that the PLM effect is based on lactate, which is present in its components. We also found that PLM increased the level of TGF-β_3, which may be associated with the prevention of the development of hypertrophic scar in the burn wound, in the blood and burn tissue during the study. We believe this may be related to less development of hypertrophic scar in the burn wound treated with PLM.

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ORİJİNAL ÇALIŞMA - *ÖZET*

Pediyatrik yanık hastalarında polilaktik membran veya gümüşlü hidrofiber pansumanların kan ve dokuda interlökin-6, tümör nekrozis faktör-α, transforming büyüme faktörü-β_3 düzeylerine etkisi

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AMAÇ: Bu çalışmanın amacı, yanmış çocuklarda iki farklı yanık sargısının (gümüşlü hidrofiber [HFAg] ve polilaktik membran [PLM]), yanık sonrası gelişen enflamasyonun şiddetini gösteren önemli biyo belirteçler olan interlökin-6 (IL-6), tümör nekroz faktörü-alfa (TNF-α) ile yanık yara iyileşmesinde önemli rol oynayan dönüştürücü büyüme faktörü-β_3'nın (TGF-β_3) kanda ve dokuda tedavi sürecindeki değişim açısından incelenmesidir.
GEREÇ VE YÖNTEM: Toplam vücut yüzey alanının %25–50'si ikinci derece kısmi kalınlıkta haşlanma yanıkları olan 1–16 yaş arası çocuklar, iki tedavi grubu çalışmaya dahil edildi. Gruplardan belirli aralıklarla PLM ile ve HFAg ile pansuman yapılan hastalardan 21 günlük tedavi döneminde 3–7–14–21. günlerde kan ve doku örnekleri alındı. Sağlıklı çocuklardan bir defa kan ve deri doku örneği alınarak kontrol grubu oluşturuldu. Gruplardan alınan kan ve doku örneklerinde IL-6, TNF-α, TGF-β_3 düzeylerine bakıldı. Sonuçlar ististiksel olarak analiz edidi.
BULGULAR: PLM grubunda IL-6 ve TNF-α seviyeleri, HFAg grubundan daha erken normal düzeye ulaştı. PLM grubunda, TGF-β_3 seviyeleri diğer gruplara göre daha uzun süre yüksek kaldı.
TARTIŞMA: Bu çalışmada, PLM'nin hem sistemik hem de yanık dokusunda enflamasyonu daha erken kontrol ettiğini bulduk. Ayrıca, PLM'nin, kanda ve yanık dokusunda hipertrofik skar gelişimini engelleyen TGF-β_3 seviyesini artırdığını da bulduk.
Anahtar sözcükler: Çocuklar; gümüşlü hidrofiber; IL-6; pansuman; polilaktik membran; TNF-α; TGF-β; yanıklar.

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