DOI: JCDR/2012/4124:2069

Original Article

Surgery Section

Amniotic Membrane Dressing versus Normal Saline Dressing in Non-healing Lower Limb Ulcers: A Prospective Comparative Study at a Teaching Hospital

HANUMANTHAPPA M.B., GOPINATHAN S., RITHIN SUVARNA, GURUPRASAD RAI D., GAUTHAM SHETTY, KARAN SHETTY, SANJEEVA SHETTY, ZUHAIL NAZAR

ABSTRACT

Background and Objectives: The management of non-healing leg ulcers poses a great challenge because of their high prevalence, refractory nature and their economic consequences on the health care system. Autologous skin graft, which is the current treatment of choice, creates a wound at the donor site. Although bioengineered skin substitutes are available, they are too expensive for the routine clinical use.

The amniotic membrane (AM) drew our interest because of its successful use in ophthalmology since long and because of its properties of promoting epithelialization and granulation, infection controlling and pain reducing. Furthermore, it is cheap, easily available, easy to preserve and apply. Hence, we undertook this study to evaluate the effects and the safety of the AM dressing.

Materials and Methods: This prospective and comparative study was conducted at the A.J. Medical College Hospital, Mangalore, from Dec 2009 to Dec 2011. We studied 200 cases with chronic leg ulcers which were divided equally and randomly into the test group (which underwent the AM dressing) and the control group (which underwent the saline dressing). The inclusion criteria were: age of 18 years or older; the presence of at least one lower limb ulcer with a minimum size of 5x5cm; and no tendency for healing in the past 3 months despite conventional medical treatment. They were visually analyzed at intervals of 7, 14 and 21 days for epithelialization, percentages of granulation tissue formation, prevention of infection, exudation, and pain control.

The AM grafts were prepared from placentas which were harvested during caesarean sections. Eligible donor mothers who tested negative for HIV, Hepatitis B and C, syphilis, toxoplasmosis, and cytomegalovirus were chosen.

Results: Epithelialization was observed in 88% of the cases in the study group (in the control group, it was 52%), the percentage of the granulation tissue increased significantly from 20% to 80%, the infection rate was 13 % in the test group (it was 59% in the control group), absence of exudation was noted in 69% cases of the test group (it was noted in 29% cases in the control group) and the pain score dropped from 70 to 10. No adverse effects were observed.

Statistical Analysis Used: Chi-square and P value.

Conclusion: We conclude that the AM dressing is a safe, cheap and effective alternative method for treating non-healing leg ulcers.

Key Words: Amniotic membrane, Biological dressing, Non-healing ulcer, Leg ulcer

INTRODUCTION

The management of non-healing leg ulcers poses a great clinical challenge because of their high prevalence, refractory nature, their impact on the patients' quality of life and their economic consequences on the health care system [1]. The current treatment of choice for these recalcitrant ulcers is autologous skin graft. But this usually requires hospitalization for several days and it creates a donor wound. Commercially available allogeneic skin substitutes are too expensive for the routine clinical use [2].

The amniotic membrane (AM) is a tissue of particular interest. Its properties such as lack of immunogenicity, fluid loss controlling, pain relieving, reepithelialization and granulation and its stimulating, antiinflammatory, antifibrotic and antimicrobial properties make it an ideal biological dressing [3,4-6,7-10,11]. It has the advantage of ready availability at no extra cost to the patient [6, 12].

It has been in use in ophthalmology for a long time. Based on its success which was observed in ophthalmology, we wished to evaluate AM as a wound dressing in chronic leg ulcers. We thus undertook a prospective comparative study (AM dressing vs normal saline dressing on 100 cases each) on patients with chronic leg ulcers to evaluate the effects and the safety of the AM dressing.

MATERIALS AND METHODS

This prospective and comparative study was conducted in the Department of Surgery, AJ Institute of Medical Sciences, Mangalore, India, from Dec 2009 to Dec 2011.

Patients

A total of 200 cases were studied, which were equally and randomly divided into the control and the test groups. The patients who presented with non-healing lower limb ulcers formed the subjects for the study. Informed consent and clearance from the local ethical committee were obtained.

The inclusion criteria were: age of 18 years or older; the presence of at least one lower limb ulcer with a minimum size of 5x5cm; and no tendency for healing in the past 3 months despite conventional medical treatment.

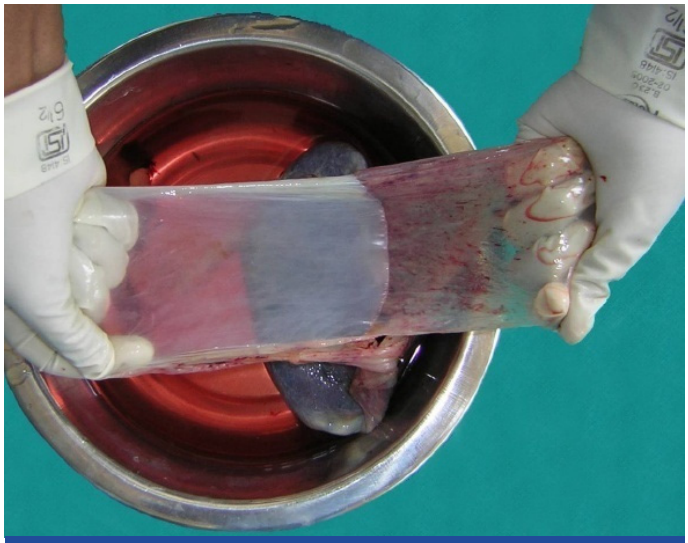
Patients with tubercular and malignant ulcers and burns were excluded from the study. Patients with severe systemic diseases and major bone exposure in the ulcer floors were also not included.

The selected patients were admitted and they underwent a detailed clinical examination. The routine haematological investigations and the culture sensitivity of the wound swab were performed for all

the cases, while the special investigations like X-ray of the part and edge biopsy were performed as and when they were required. The patients underwent treatment for a period of one to two weeks before the study to stabilize the wound and appropriate medical and surgical lines of treatment like diabetes control, control of the infection by initiating the appropriate antibiotic based on the culture sensitivity report, surgical debridement, and correction of the medical illness were carried out during this period.

Once the ulcers showed signs of granulation tissue, they were subjected to the study. Prior to the study, a repeat culture swab was taken from each ulcer. *Streptococci*, if present, were treated with appropriate antibiotics and the patients were then subjected to the study when their cultures showed no growth. Then, the eligible patients were divided randomly into the test and the control groups.

Amniotic membrane (harvesting, preservation and its application):



[Table/Fig-1]: Peeling of the amniotic membrane from the placenta

The AM grafts were prepared from placentas which were harvested during caesarean sections. Eligible donor mothers were accepted for the AM donation after a medical interview and after a written informed consent was obtained from them. Their blood samples were tested for HIV, Hepatitis B and C, *syphilis*, *toxoplasmosis*, and *cytomegalovirus* [13]. Those who tested negative, with no premature rupture of the membranes, were chosen for the donation.

The AMs were separated from the chorions of the placentas under sterile aseptic conditions [Table/Fig-1]. The AMs were cleared of all gross tissue attachments and blood clots by washing them in copious amounts of normal saline. The membranes were then placed in large bottles which contained 85% glycerol and they were stored at room temperature for 24 hours and then at 4°C in the refrigerator until use. The membranes were tested for bacterial count and culture sensitivity prior to their use. At the time of application, the AMs were thawed by soaking them in normal saline for 10 minutes. They were then spread over the surface of the ulcers and a non-occlusive dressing was placed over them.

Method of application of the dressing:

Test group: The ulcers were cleaned and irrigated with saline, the AMs were applied with their rough (chorionic) surfaces facing the surface of the ulcers and a 3 layered gauze dressing was placed [14,15]. The dressing was left in place for 4 days and it was observed for any exudation. Thereafter, a redressing was done once in 3 days and it was evaluated on the 7th, 14th and 21st days.

Control group: The ulcers were cleaned and subjected to normal saline dressing once or twice daily, depending on the exudates.

Method of evaluation of the wound:

At the end of the 1st, 2nd and 3rd weeks, the test group (AM dressing) and the control group (normal saline dressing) were evaluated and compared. The parameters which were recorded at each evaluation were epithelialization of the ulcer, percentages of granulation tissue, the local pain score, exudation and prevention of wound infection.

The local pain score was assessed by using a 101-point (0–100) visual analogue scale, with 0 indicating no pain and 100 indicating the worst pain which was imaginable.

A foul smelling purulent discharge, any change in colour of AM and surrounding erythema with local signs of inflammation were taken as suggestive of an infection. If the 3 layered gauze dressing was soaked, exudation was considered to be present.

Statistical analysis used: Chi square test and P value.

The results were analyzed and conclusion were drawn.

RESULTS

The clinical details of the cases which were studied have been shown in [Table/Fig-2].

Feature	Test group (N=100)	Control group (N=100)
Male	82	85
Female	18	15
Mean age in years (range)	48 (18-78)	47 (18-76)
Mean duration of ulcer in months (range)	5 (3-7)	4.5 (3-6)
Ulcer types:		
Ischemic ulcer	19	17
Neuropathic ulcers	28	23
Venous ulcers	12	10
Post-traumatic ulcer	34	42
Others	7	8
Co-morbidity:		
DM	36	30
Cardiac diseases	24	21
Others	6	5

[Table/Fig-2]: Clinical details of test & control group

In total, 100 AM grafts were applied on 100 chronic lower limb ulcers. At the end of the 1st, 2nd and 3rd weeks, the test group (AM dressing) and the control group (normal saline dressing) were evaluated and compared. The parameters which were compared were epithelialization of the ulcer, percentages of granulation tissue, pain control, exudation and prevention of infection.

Epithelialisation:

Epithelialisation	Control group (N=100)	Test group (N=100)
At 1st week end	0	18
At 2nd week end	23	45
At 3rd week end	31	25
No epithelialisation	46	12

[Table/Fig-3]: Epithelialisation
Chi-squared=45.692 P< .001 Statistically significant

Out of 100 cases in the test group, 88 (88%) cases showed epithelialization by the end of the 3rd week as compared to 52 (52%) in control group [Table/Fig-3].

Most of the cases, which showed complete epithelialization at the end of the 1st week were young with traumatic ulcers, without co-morbid conditions like diabetes. In 45 cases, complete epithelialization was observed at the end of the 2nd week. In this group, a majority of the ulcers were traumatic, ischaemic and venous ulcers with or without well controlled diabetes mellitus. In elderly patients and in cases with uncontrolled diabetes mellitus, the epithelialization was delayed.

Wound infection:

Infection	Control group (N=100)	Test group (N=100)
Absent	41	87
Present	59	13

[Table/Fig-4]: Comparision of infection rate
Chi-squared=45.92 P< .001 Statistically significant

The AM prevented wound infections in 87% of the cases against 41% in the control group [Table/Fig-4].

Exudation:

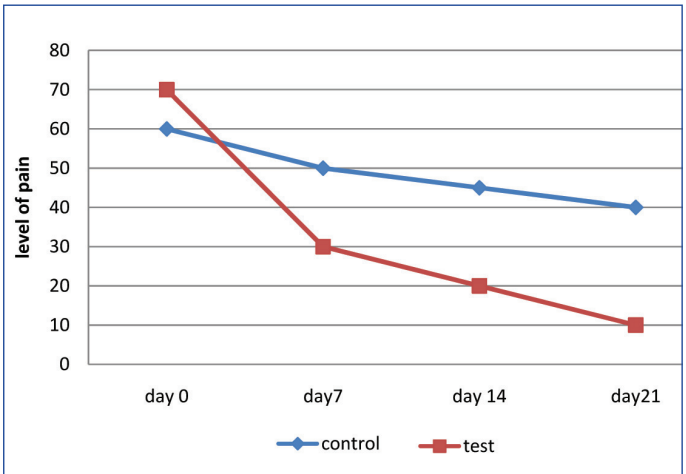
Exudation	Control group (N=100)	Test group (N=100)
Absent	29	69
Present	71	31

[Table/Fig-5]: Exudation control by AM
Chi-squared=57.758 P-value > .0001 Statistically highly significant

By the end of the 1st week, the dressings were found to be dry in 69 (69%) cases in the test group as compared to 29% in the control group [Table/Fig-5].

Pain relief:

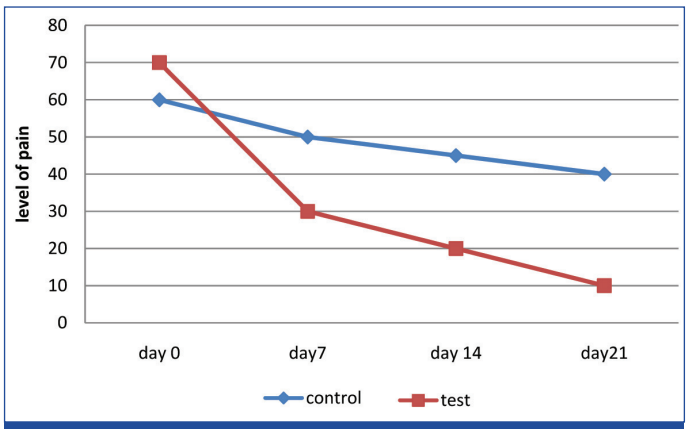
The pain score in the test group dropped from 70 to 10 in 3 week's time. A maximal effect was observed in the first week [Table/Fig-6].



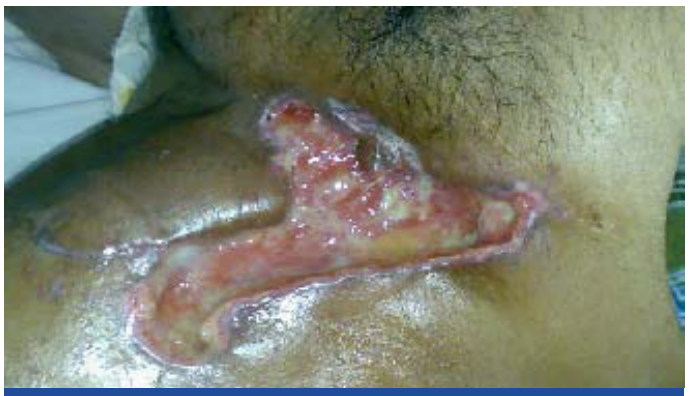
[Table/Fig-6]: Pain relieveing property of AM

Granulation:

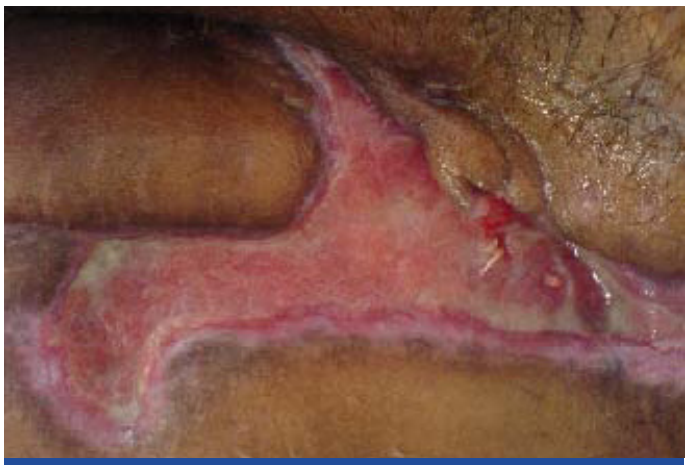
The %age of the granulation tissue increased significantly in the test



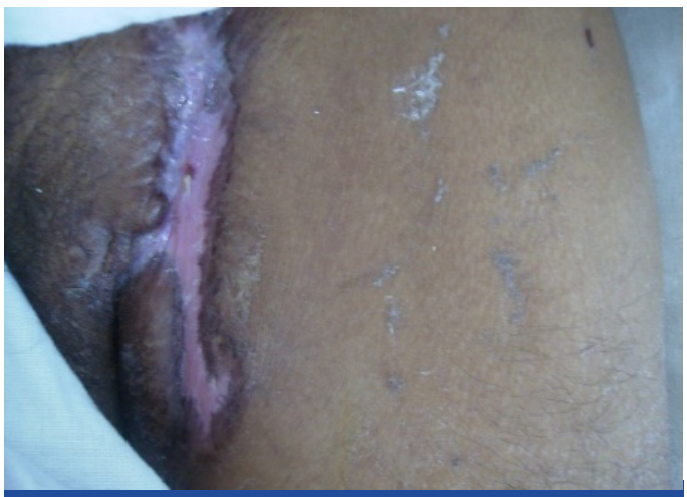
[Table/Fig-7]: Granulation stimulating effect of AM



[Table/Fig-8]: Non healing groin ulcer with exudation (before Application of amniotic membrane dressing)



[Table/Fig-9]: 7th day after AM dressing; No exudation



[Table/Fig-10]: After 3 weeks of AM dressing; healed ulcer

group from 20% (mean) to 80% (mean) in 3 weeks as compared to the control group [Table/Fig-7]. A maximal effect was observed in the first 2 weeks in the test group. It was healthy and vascular.

DISCUSSION

Human amniotic membrane (AM):

The amnion is the inner most lining of the foetal membranes. It is made up of two membranes, the inner amniotic membrane and the outer chorion. The AM can be easily separated from the chorion. The AM is a thin but tough, smooth and transparent membrane.

As a biological dressing, it has the following properties:

- It provides secure coverage to the wound site, which reduces exudation from the wound.

Antimicrobial property:

This property is believed to be due to the presence of antibodies in the AM and the impervious nature of the AM to micro-organisms [1]. The high thrombin activity of the AM allows a very rapid and efficient attachment of the AM to the granulating surface [16]. This close adherence eliminates the exposed status of the wound, which checks the bacterial count and allows restoration of the lymphatic integrity, which protects the circulating phagocytes from exposure and allows the removal of the surface debris and the bacteria [1, 3].

- The AM stimulates epithelialization from the ulcer bed and/or the wound edge, which is considered to be mediated by growth factors and progenitor cells which are released by it [15, 14].
- One of the most striking effects, as was noted by Faulk et al and Burgos, is its granulation stimulating effect. This is due to some angiogenic and growth factors which are produced by the membrane [16, 7-10].
- Despite being a human derivative, it is not rejected, because the AM does not express the HLA A,B,C and the DR antigens, as was stated by Ward and Bennet in their study [7].

Pain Relief:

This is one of the well recognized properties of the AM when it is used as a skin substitute [17,14]. This is possibly due to the diminished inflammation, the better state of hydration of the wound bed [18,19] and protection of the exposed nerve endings from external irritants.

- Its other important properties are its anti-adhesive property (it peels off on its own once the surface is epithelialized) and its scar reducing property [20].

Various techniques and methods have been described for its preservation. We followed the glycerol preservation method, because of the ease of preservation and reconstitution, low cost and because of the anti bacterial and antiviral properties of glycerol. A glycerol preserved amnion is as effective as a fresh amnion [6].

Another Indian study which used 85% glycerol for amnion preservation showed excellent results which were obtained with the use of this extremely economical dressing. This emphasizes the importance of establishing “amnion banks” in all hospitals, especially in the developing countries [6].

Method of use:

Before the membrane is applied, the wound should be prepared as it is prepared for skin grafting. A surgical scrub with antiseptic and minimal debridement is followed by moist compression until the oozing has stopped and the wound surface is reasonably dry. This procedure is preferably done in a clean, sterile dressing room, with observation of all the aseptic measures [16]. The membrane is applied with its rough (chorionic) surface next to the wound [16]. Care is taken to ensure that there is no trapping of air bubbles between the membrane and the wound by gently pressing it. The membrane is followed by a layer of anti-bacterial gauze (e.g. Soframycin tulle), some moist gauze, dry gauze, cotton and bandage.

CONCLUSION

We conclude that the amniotic membrane dressing is a safe, cheap and effective alternative method for treating non-healing leg ulcers, particularly in developing countries, where the cost of the dressing material is the major concern.

As India is a developing country with a vast population and an exorbitant requirement of wound care resources, “amniotic membrane banks” at every hospital could be an answer!

ACKNOWLEDGEMENT

We would like to thank all the consultant surgeons at the AJ Institute of Medical Sciences, Mangalore, for allowing us to analyze their cases. We thank Dr. Nanjesh for his help with the statistical analysis. The authors confirm that there are no known conflicts of interest which are associated with this publication and that there has been no financial support for this work that could have influenced its outcome.

REFERENCES

[1] Patel NP, Labropoulos N, Pappas PJ. Current management of venous ulceration. Plast Reconstr Surg 2006; 117: 254–60.
[2] Horch RE, Kopp J, Kneser U, Beier J, Bach AD. Tissue engineering of cultured skin substitutes. J Cell Mol Med 2005; 9: 592–608.
[3] Quinby WC, Hoover HC, Scheflan M, et al. Clinical trials of amniotic membranes in burn wound care. Plast Reconstr Surg 1982; 70:711.
[4] Faulk WP, Matthews RN, Stevens PJ, et al. Human amnion as an adjunct in wound healing. Lancet 1980; 1:1156.
[5] Bennett JP, Matthews RN, Faulk WP. Treatment of chronic ulceration of the legs with human amnion. Lancet 1980; 1:1153.
[6] Ravishanker R, Bath AS, Roy R. ‘Amnion Bank’ - use of long term glycerol preserved amniotic membrane for the management of superficial and superficial partial thickness burns. Burns 2003; 29: 369-74. [PUBMED].
[7] Akle A.C, Adinolfi M, Welsh KI, Leibowitz S, Mccoll I. Immunogenicity of the human amniotic epithelial cells after their transplantation into volunteers. Lancet 1981; 1:1003.
[8] Kesting MR, Wolff KD, et al. The role of allogenic membranes in burn treatment. J Burn Care Res 2008; 29(6):907-16.
[9] Lineen E, Namias N. Biologic dressing in burns. J Craniofac surg 2008; 19(4):923-8.
[10] Salisbury RE, Carnes RW, et al .Biological dressings and evaporative water loss from burn wounds. Ann Plast Surg 1980; 5(4):270-2.
[11] Insausti CL, Alcaraz A, García-Vizcaíno EM, Mrowiec A, López-Martínez MC, et al. Amniotic membrane induces epithelialization in massive posttraumatic wounds. Wound Repair and Regeneration 2010; 18: 368–77.
[12] Torrati FG, Rossi GA, Ferreira E, et al. Analysis of the cost of dressings in the care of burns patients. Burns 2000; 26:289-93.
[13] Mermet I, Pottier N, Sainthillier JM, Malugani, Cairey-Remonnay S, et al. Use of amniotic membrane transplantation in the treatment of venous leg ulcers. Wound Rep Reg 2007; 15: 459–64.
[14] Kasi N, Durrani KM, Siddiqui MA. Human amniotic membrane as a versatile biological dressing. A preliminary report. J Pak Med Assoc 1987; 37:290.
[15] Bailo M, Soncini M, Vertua E, Signoroni PB, Sanzone S, Lombardi

G, et al. Engraftment potential of the human amnion and the chorion cells which are derived from term placenta. Transplantation 2004; 78: 439–48.
[16] Ganatra MA. The amniotic membrane in surgery. JPMA Jan 2003.
[17] Sharma SC, Bagree MM, Bhat, et al. Amniotic membrane is an effective burn dressing material. Jpn J Surg 1985; 15/2:140.
[18] Landi G, Fortuna A, Mengozzi E, at al. Medication burns with the amniotic membrane. G Ital Dermatol/Minerva Dermatol 1977; 112:343
[19] Rao TV, Chandrasekharam V. Human amnion as a dressing material in burns. Indian J Surg 1981; 43:561.
[20] Rejzek A, Weyer F, Eichberger R, Gebhart W. Physical changes of amniotic membranes through glycerolization for their use as an epidermal substitute. Light and electron microscopic studies. Cell Tissue Bank 2001; 2(2):95-102.

AUTHOR(S):
1. Dr. Hanumanthappa M.B.
2. Dr. Gopinathan S.
3. Dr. Rithin Suvarna
4. Dr. Guruprasad Rai D.
5. Dr. Gautham Shetty,
6. Dr. Karan Shetty
7. Dr. Sanjeeva Shetty
8. Dr. Zuhail Nazar

PARTICULARS OF CONTRIBUTORS:
1. Corresponding Author,
2. Assistant Professor, Department of Surgery,
3. Associate Professor, Department of Surgery,
4. Resident, Department of Surgery,
5. Resident, Department of Surgery,
6. Resident, Department of Surgery,
7. Resident, Department of Surgery,
8. Resident, Department of Surgery,

AJ Institute of Medical Sciences, Mangalore, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Hanumanthappa M.B.
AJ Institute of Medical Sciences, Department of Surgery,
Mangalore, India - 575004.
Phone: 9845170266
E-mail: mb.hanumanthappa@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS:
None.

Date of Submission: **Feb 09, 2012**
Date of Peer Review: **Mar 04, 2012**
Date of Acceptance: **Mar 22, 2012**
Date of Publishing: **May 01, 2012**