

## EFFECTS OF NATURAL ORIFICE SECRETIONS IN PERITONEAL CAVITY IN THE BACKGROUND OF NATURAL ORIFICE TRANSLUMINAL ENDOSCOPIC SURGERY (NOTES) AN EXPERIMENTAL STUDY IN ANIMALS

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### HOW TO CITE THIS ARTICLE:

Devendra Chaudhary, Arvind Rai, R. S. Gupta, Dipendra Pradhan, Anuradha Chaudhary, Rohit Namdev. "Effects of Natural Orifice Secretions in Peritoneal Cavity in the background of Natural Orifice Transluminal Endoscopic Surgery (Notes) an Experimental study in Animals". Journal of Evidence based Medicine and Healthcare; Volume 2, Issue 10, March 09, 2015; Page: 1509-1518.

**ABSTRACT:** Natural Orifice Transluminal Endoscopic Surgery (NOTES) is a new form of minimally invasive surgery which eliminates traditional skin incisions by accessing internal body cavities through natural orifices. In our experimental animal study, we compared the incidences intraperitoneal abscess formation, culture swab of peritoneal cavity positive for organism, intraperitoneal adhesion formation and mean adhesion score before and after lavaging the portal of entry of albino rat, i.e. transgastric and transvaginal. On vaginal route as a portal of entry into peritoneal cavity, on the 7th day, 66% rats developed abscesses, 88% rats had culture swab positive and 88% rats developed intraperitoneal adhesion (grade-2) before any cleansing of vaginal cavity with antiseptic solution. Now after lavage with povidone iodine solution, only 11% developed abscesses, 22% were peritoneal swab culture positive and 33% had interbowel and parietal adhesion of (grade 0-1). On 21<sup>st</sup> day, the complication observed was adhesion formation in pre lavage group of 66% incidence and 16% after vaginal lavage. The incidence of complications were reduced significantly after lavage with antiseptic solution as shown by p values ( $p < 0.01$  for abscess formation,  $p < 0.01$  for culture positivity and  $p < 0.01$  for adhesions formation). Also the mean adhesion scoring was significantly reduced ( $p < 0.02$ ) after vaginal lavage on the 7<sup>th</sup> day. Gastric route as the portal of entry into the peritoneal cavity, again the same variables were compared on the 7th and the 21st day, but wash was given with antibiotic solution (Cefazolin). On the 7th day, 44% had abscesses, 77% were culture positive and 66% had adhesions (Grade 1-2) before gastric lavage with antibiotic solution. After wash of stomach, 11% were culture positive and 44% developed adhesions (Grade 0-1). Here, abscess formation ( $p < 0.02$ ) and mean adhesion scoring ( $p < 0.05$ ) were significantly reduced after stomach wash. On the 21st day, the incidences of complications were markedly decreased in the post-gastric lavage group. In the group without antibiotic lavage, incidences for adhesions was 50%, culture positivity was 33% and mean adhesion score was 2.66. In the post lavage group, 20% developed adhesions.

**INTRODUCTION:** Natural Orifice Transluminal Endoscopic Surgery (NOTES) is a new form of minimally invasive surgery which eliminates traditional skin incisions by accessing internal body cavities through natural orifices such as the mouth, anus, transvaginal, transvesical and nasal. The first report of oral peritoneoscopy done in animal was published by kallo et al<sup>(1)</sup> in 2004 since then, multiple investigator have used transilluminal flexible endoscopy in animal to perform various intraperitoneal procedure, ranging from tubal ligation to splenectomy.<sup>(1-6)</sup>

# ORIGINAL ARTICLE

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Natural Orifice Transluminal Endoscopic Surgery (NOTES) can be further defined as Transluminal surgery (TS) and Endolumenal surgery (ES). However, several important issues, including the safety of this approach and whether it will provide significant patient benefit in terms of postoperative recovery compared with laparoscopy, must be resolved before the new technique is widely introduced into clinical use.

NOTES may have several advantages in specific subpopulations. It may provide an easy alternative access to the peritoneal cavity in morbidly obese patients, in whom traditional open or laparoscopic access can be difficult because of abdominal wall thickness. NOTES can also offer diagnostic and therapeutic measures to moribund ICU patients and can reduce unnecessary invasive interventions.<sup>(7)</sup>

Benefits of laparoscopic surgery also apply to NOTES which include decreased neurohumoral stress response, decreased immunosuppression<sup>(8)</sup>, less pain faster recovery, and a decreased incidence of wound-related and pulmonary complications.<sup>(9)</sup>

The incidence of incisional hernia is substantially lower with laparoscopic procedures, where incision size is much smaller than for open surgery, and should be eliminated with NOTES (4%- 8% with open surgery<sup>(10-12)</sup> vs 0.2%~3% with laparoscopic surgery).<sup>(13)</sup>

The reported rates of small-bowel obstruction due to adhesions are also significantly lower after laparoscopic surgery compared with open surgery (3.3% vs 7.7%) and will perhaps be further decreased with NOTES.<sup>(14)</sup>

Although NOTES appears to offer definite patient benefits, several critical issues must be resolved before it can be successfully and responsibly used in clinical care. Additionally, it is recognized that early use of this approach by surgeons or gastroenterologists who might be relatively inexperienced in this field, requires particular skills otherwise it may lead to serious complications, which should definitely be avoided during the infancy of this concept.

In this study, we have handpicked two problems viz. infection rate and adhesion formation in peritoneal cavity of albino rats after contact with natural orificial secretions. We also compared the incidences of infection rate and adhesion formation before and after lavaging the portal of entry, i.e. transgastric and transvaginal.

This study on rats tries to address dilemmas not only on the infective but also the physiological effects of natural orifice secretions of two important routes (transgastric and transvaginal) in the peritoneal cavity.

**AIMS AND OBJECTIVES:** To study complications of gastric aspirate and vaginal fluid inoculated into peritoneal cavity with or without lavage with antibiotic/antiseptic solution, and document differences in the incidences of complications in peritoneal cavity in rats viz. abscess formation, peritoneal swab culture, adhesions formation, in both pre and post lavage groups in the context of NOTES (Natural Orifice Transluminal Endoscopic Surgery).

**MATERIAL & METHODS:** The present study "Effects of natural orifice secretions in peritoneal cavity in the background of NOTES: an experimental study in animals" was carried out in laboratory of pharmacology department of Gandhi Medical College, Bhopal (M.P.) India.

60 Albino rats were used in this study. This included animals of either sex and were kept with labels in respective animal cages, noting their weight, category of experiment (test & control), date and number of experiment. The animals were kept at room temperature and

# ORIGINAL ARTICLE

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provided free access to standard (Bengal gram) green leafy vegetables, bread and tap water prior to the experiment.

**STUDY DESIGN:** A prospective randomized controlled study was conducted on 60 rats to look for the effects of gastric aspirate and vaginal lavage fluid in the peritoneal cavity. Laparotomy was done on the 7<sup>th</sup> day and the 21<sup>st</sup> day to look for signs of bacterial peritonitis and intra abdominal adhesions.

**ANESTHESIA:** Ether was used for induction as well as to maintain the anesthesia.

**PROCEDURE:** The animals included in the study were adults rats, of appropriate weight (150-250 gms), assessed for its vitality and kept nil by mouth for 4 hours before the experiment.

- A. The rats were randomly divided into 5 groups of equal number.
  1. GROUP Ia: the test group inoculated with gastric aspirate before lavage of stomach with antibiotic solution.
  2. GROUP Ib: the test group inoculated with gastric aspirate after lavage of stomach with antibiotic solution
  3. GROUP IIa: the test group inoculated with vaginal lavage fluid before lavage of vagina with antiseptic solution.
  4. GROUP IIb: the test group inoculated with vaginal lavage fluid after lavage of vagina with antiseptic solution.
  5. GROUP III: the control group inoculated with normal saline.
- B. After appropriate anaesthesia, for group Ia, feeding tube of appropriate sizes was inserted into the stomach through oral route and gastric aspirate withdrawn without any lavage of stomach with antibiotic solution.
- C. For group Ib, after insertion of the feeding tube, first a lavage with cefazolin solution was done through the feeding tube and after time interval of 3 mins, again gastric aspirate was aspirated.
- D. The gastric aspirates which was approximate 1-4 ml in quantity was then inoculated into the peritoneal cavity in right iliac fossa (RIF) region after achieving sterility of the skin and lifting of the parietal wall so as not to perforate the bowel.
- E. For group IIa, first, the genital area was cleaned with antiseptic solution (povidone- iodine) and vagina irrigated with normal saline filled in 2 ml syringe and this irrigation fluid subjected to experiment, i.e. inoculated into the peritoneal cavity in RIF region as described above.
- F. For group IIb, initially lavage of the vagina was done with antiseptic solution (povidone solution) and after 3 mins, repeat irrigation of the vagina done with normal saline and this irrigation fluid was subjected to experiment.
- G. For group III which is the control group, simply 1-3 ml of normal saline was injected into the peritoneal cavity of animals.
- H. All the rats in each groups were kept in separate cages with proper labels for identification. On the 7<sup>th</sup> day, half the number of rats from each group was subjected to laparotomy under sterile condition. Peritoneal cavity was thoroughly inspected for signs and site of peritonitis (pus, adhesions, abscesses) and peritoneal swab taken for bacteriological culture. Adhesions

# ORIGINAL ARTICLE

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and the incidence of abscesses were examined in a blinded manner according to the method of Zuhlke et al.<sup>(15)</sup>

Sites of adhesions scored included the right iliac region, pelvic region, the upper abdomen (liver), the parietal peritoneum, the omentum, and between the bowel loops. The total score of these 6 locations was noted as the total adhesion score (0-24).

- I. On the 21st day, remaining half of animals in each group was subjected to laparotomy and procedure repeated as above.
- J. The observation was tabulated and tested for significance.

**Bacterial Cultures:** Samples of peritoneal fluid and abscesses were taken from all rats in the experiment on the 7<sup>th</sup> and 21<sup>st</sup> day of inoculation. The swabs were immediately introduced into medium and cultured semi quantitatively in aerobic and anaerobic conditions. Samples were incubated on blood and Eosin methylene blue agar for aerobic culture and layered on anaerobic blood agar and incubated in a Gas-Pak jar for anaerobic culture. After 24 and 48 hours of incubation at 37°C, growing colonies were identified with standard bacteriologic techniques.

## **GRADING OF ADHESIONS ACCORDING TO ZUHLKE ET AL:<sup>(15)</sup>**

**OBSERVATION & RESULTS:** Taking into consideration the vaginal route as an access portal to peritoneal cavity, and the effects of its secretion in the peritoneal cavity, four variables were compared before and after the lavage with antiseptic solution (povidone solution) of the vaginal cavity, viz. intraperitoneal abscess formation, culture swab of peritoneal cavity positive for organism, intraperitoneal adhesion formation and mean adhesion score. These comparisons were done for different sets of rats on the 7<sup>th</sup> day and the 21<sup>st</sup> day.

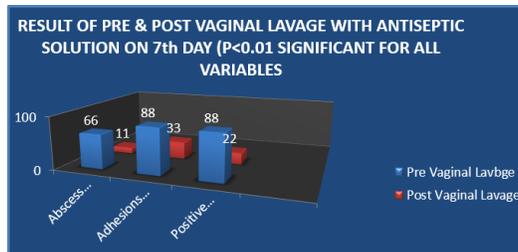
On the 7<sup>th</sup> day, 66% rats developed abscesses, 88% rats had culture swab positive and 88% rats developed intraperitoneal adhesion (grade 1-2) before any cleansing of vaginal cavity with antiseptic solution. Now after lavage with povidone iodine solution, only 11% developed abscesses, 22% were peritoneal swab culture positive and 33% had interbowel and parietal adhesion of (grade 0-1).

Definitely, the incidence of complications were reduced significantly after lavage with antiseptic solution as shown by p values ( $p < 0.01$  for abscess formation,  $p < 0.01$  for culture positivity and  $p < 0.01$  for adhesions formation). Also the mean adhesion scoring was significantly reduced ( $p < 0.02$ ) after vaginal lavage on the 7<sup>th</sup> day.

Or the gastric route as the portal of entry into the peritoneal cavity, again the same variables as mentioned above were compared on the 7<sup>th</sup> and the 21<sup>st</sup> day, but here the wash was given with antibiotic solution (Cefazolin).

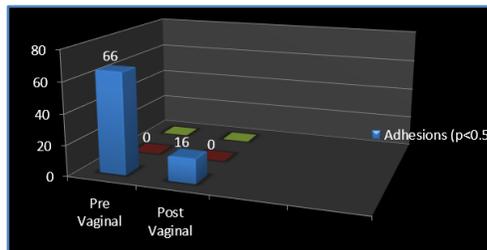
On the 7<sup>th</sup> day, 44% had abscesses, 77% were culture positive and 66% had adhesions (grade 1-2) before gastric lavage with antibiotic solution. After wash of stomach, 11% were culture positive and 44% developed adhesions (grade 0-1). Here, abscess formation ( $p < 0.02$ ) and mean adhesion scoring ( $p < 0.05$ ) were significantly reduced after stomach wash.

# ORIGINAL ARTICLE



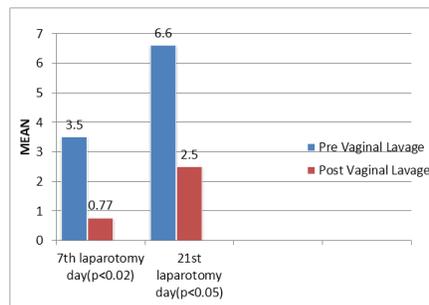
**Figure 1**

**Fig. 2:** Results of Pre & Post Vaginal Lavage with Antiseptic Solution on 21<sup>st</sup> Day (Significant for Adhesions).



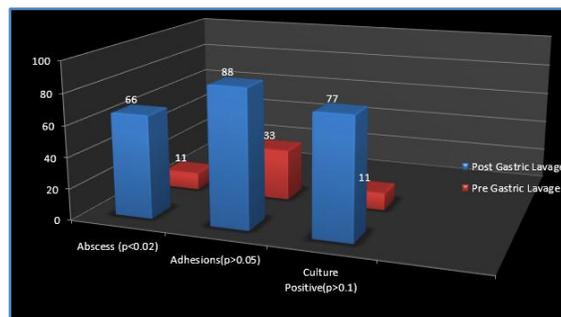
**Figure 2**

**Fig. 3:** Comparison of Mean of Adhesion Score in Pre and Post Vaginal Lavage with Antiseptic Solution.



**Figure 3**

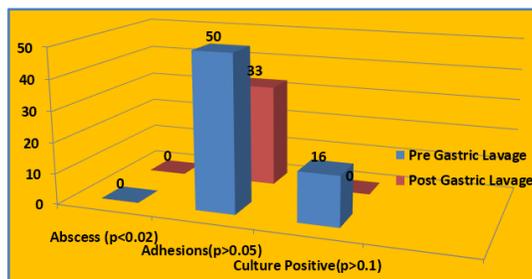
**Fig. 4:** Results of Pre and Post Gastric Lavage with Antibiotic Solution on 7<sup>th</sup> day (Significant for Abscess Formation Only).



**Figure 4**

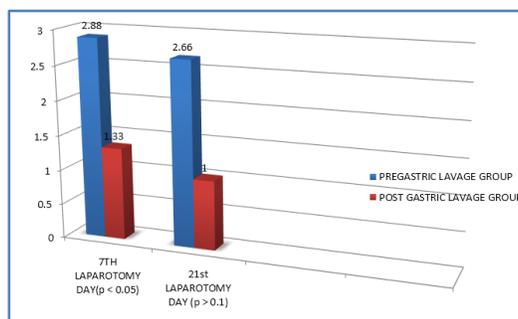
# ORIGINAL ARTICLE

**Fig. 5:** Result of Pre & Post Gastric Lavage with Antibiotic Solution On 21<sup>st</sup> Day.



**Figure 5**

**Fig. 6:** Comparison of Mean of Adhesion Score in Pre and Post Gastric Lavage with Antibiotic Solution.



**Figure 6**

**DISCUSSION:** A new dimension has now been added to minimally invasive surgery, with the advent of natural orifice transluminal endoscopic surgery (NOTES).

The entire premise of transluminal surgery is based on the potential advantages associated with scarless access to the peritoneal cavity. So far, however, there have been very few studies to show exactly what the advantages are. Studies needed to demonstrate the efficacy of NOTES in preventing postoperative hernias, infective complications, adhesions and abdominal wall pain would be cumbersome and require years of follow-up.

Until the issues surrounding the advantages of NOTES are addressed, the potential benefits of transluminal surgery remain theoretical and without a scientific basis. On the other hand, laparoscopic surgery has a proven track record and is associated with minimal morbidity and mortality.

Some complications are to be expected with any procedure, but there should be a low threshold for tolerating complications arising from NOTES procedures. A policy of zero or low-tolerance for complications that are unique to the method (e.g. peritoneal infection, bleeding and damage to surrounding structures related to gastrotomy and its closure) must, therefore, be a priority when translating NOTES to human use.

Our concerns, as reflected in this study were to see how a virgin surface or cavity behaves to secretions from natural orifices in rats keeping NOTES in mind. Here, the virgin surface is the peritoneal cavity. The vagina becomes colonized soon after birth with corynebacteria,

## ORIGINAL ARTICLE

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staphylococci, streptococci, E. coli, and a lactic acid bacterium historically named "Doderlein's bacillus" (*Lactobacillus acidophilus*). In the upper GI tract of adult humans, the esophagus contains only the bacteria swallowed with saliva and food. Because of the high acidity of the gastric juice, very few bacteria (mainly acid-tolerant lactobacilli) can be cultured from the normal stomach most important of which is the *Helicobacter pylori*.<sup>(15)</sup> The proximal small intestine has a relatively sparse Gram-positive flora, consisting mainly of lactobacilli and *Enterococcus faecalis*. This region has about  $10^5$ - $10^7$  bacteria per ml of fluid. The distal part of the small intestine contains greater numbers of bacteria ( $10^8$ /ml) and additional species, including coliforms (*E. coli* and relatives) and *Bacteroides*, in addition to lactobacilli and enterococci.

Secondary peritonitis is, by far, the most common form of peritonitis encountered in clinical practice today. It is caused by perforation or necrosis (transmural infection) of a hollow visceral organ with bacterial inoculation of the peritoneal cavity. Gram-positive organisms predominate in the upper GI tract; however, a shift toward gram-negative organisms may be noticed in patients on long-term gastric acid suppressive therapy.

**ASSESSMENT OF GROUP WITH INOCULATION OF GASTRIC ASPIRATE INTO PERITONEAL CAVITY:** The rat received gastric lavage with cefazolin solution and saline lavage and aspirate was retrieved through feeding tube. The aspirate was inoculated into the peritoneal cavity in the right iliac fossa region. Sterile laparotomy was done on 7<sup>th</sup> and on 21<sup>st</sup> day.

On the 7<sup>th</sup> day in saline lavage group, 44% rats had abscess, 77% were culture positive and 66% had adhesion. In the group lavaged with cefazolin solution, no rats had abscess, 11% were culture positive and 44% developed adhesion. On comparing these two groups, the incidence of abscess formation ( $p < 0.02$ ) and mean adhesion scoring ( $p < 0.05$ ) was reduced significantly after gastric lavage with cefazolin solution. It may be noted here that in spite of lowered rates of complication after antibiotic wash, percentage of adhesion formation was still higher in post gastric wash group. From this it may be inferred that beside infective aspect, chemical peritonitis also had role in adhesion formation.

The mean adhesion score on the 7<sup>th</sup> day for group without antibiotic gastric lavage was 2.88 whereas in post gastric lavage group was 1.33. On comparing the mean adhesion scores of two by student t test, p value was  $< 0.05$  which is highly significant.

But, Mc Gee MF et al (Cleveland)<sup>(17)</sup> in a related study, conducted experiment in 20 pigs. The pigs received gastric lavage with saline solution, chloramphenicol, or no lavage, and then underwent transgastric notes peritoneoscopy. The animal was observed for 14 days and underwent sterile laparotomy. 19 animals underwent NOTES: 18(94.7%) survived the entire postoperative period. ONE animal died on postoperative day 2 after the peg tube dislodged at 14 days, 5 animals (27.8%) had intraabdominal abscesses, 8(44.4%) had positive peritoneal cultures, and 9(50%) foreign bodies were contaminated on culture. Infectious complications were not altered by the type of gastric lavage or peritoneal bacterial inoculum at the time of surgery.

In another study by Narula VK et al<sup>(18)</sup> in 50 humans, to quantitate the colony forming units (cfu) in peritoneal cavity before and after gastrostomy, he concluded that trans gastric instrumentation does contaminate the abdominal cavity but pathogens are clinically insignificant due to species or bacterial load. Patients on PPI do have an increased bacterial load in the gastric aspirate, with no clinical significant infection.

# ORIGINAL ARTICLE

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Mohamed A Babatin in his study published in Saudi journal of gastroenterology <sup>(19)</sup>, stated that the clinical experience of bacteriological contamination of the peritoneum derived from decades of bowel surgery suggests that it is well tolerated, provided gross spillage is avoided and patients receive prophylactic antibiotics. In the animal studies published thus far, maneuvers to sterilize the stomach prior to the procedure and the use of a sterile over tube seem to have reduced risk of developing intraperitoneal abscesses. It is therefore feasible that infection may not pose additional difficulties in harnessing this technique further.

On the 21<sup>st</sup> day, the incidences of complications were markedly decreased in the post gastric lavage group. In the group without antibiotic lavage, incidences for adhesions was 50%, culture positivity was 33% and mean adhesion score was 2.66. In the post lavage group, 20% developed adhesions.

In contradiction to above studies by Mc Gee MF et al (Cleveland), <sup>(17)</sup> by Narula VK et al, <sup>(18)</sup> which postulates that: 'Infectious complications were not altered by the type of gastric lavage or peritoneal bacterial inoculum introduced at the time of surgery', our study observed reduced incidences of complications after antibiotic/ antiseptic solution lavage.

**ASSESSMENT OF GROUP WITH INOCULATION OF VAGINAL LAVAGE FLUID INTO PERITONEAL CAVITY:** on the 7<sup>th</sup> day: in saline lavage group, 66% rats had abscess, 88% were culture positive and 88% had adhesions. In the group, with lavage with povidone iodine solution, 11% rats had abscess, 22% were culture positive and 33% developed adhesions. On comparing the two groups, the incidence of abscess formation, positive culture and adhesion formation ( $p < 0.01$ ) and mean adhesion scoring ( $p < 0.02$ ) was reduced significantly after vaginal lavage with povidone iodine.

On 21<sup>st</sup> day, the complication observed was adhesion formation in pre lavage group of 66% incidence and 16% after vaginal lavage. Thus, it can be inferred from this study that no route is safe and devoid of complications. The magnitude and the severity of complications can be reduced by adequate preparation of natural orifices before any kind of intervention through it. This will be more obvious if we consider Trans colonic route of access to peritoneal cavity which is flourished with organisms.

**CONCLUSION:** In our study the incidence of adhesion brought down from 66% to 16% after antiseptic lavage. Similarly on 7<sup>th</sup> day, the incidence of adhesion, abscess formation and positive culture were reduced by 22%, 44% and 66% respectively after lavage of stomach with antibiotic solution.

In transvaginal route as a portal of entry on the 7<sup>th</sup> day, 66% rats developed abscesses, 88% rats had culture swab positive and 88% rats developed intraperitoneal adhesion (grade i-2) before any cleansing of vaginal cavity with antiseptic solution. Now after lavage with povidone iodine solution, only 11% developed abscesses, 22% were peritoneal swab culture positive and 33% had interbowel and parietal adhesion of (grade 0-1).

On 21<sup>st</sup> day of inoculation the incidence of adhesion formation was 50% in pre antibiotic lavage group against 33% after gastric lavage with antibiotic solution. From the results obtained, it may be inferred that, transgastric and transvaginal routes as portal of access to advance into peritoneal cavity, are not devoid of complication altogether. But simple measures like pre-

# ORIGINAL ARTICLE

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procedural wash of natural orifices with antiseptic or antibiotic solution can minimize the risk of complications. Further well managed human studies need to be conducted to determine the safety and efficacy of notes in clinical setting.

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Date of Submission: 23/11/2015.  
Date of Peer Review: 24/11/2015.  
Date of Acceptance: 04/03/2015.  
Date of Publishing: 06/03/2015.