

1 Details of your submission

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Title: Characterization of major constituents of biofilms adhering to indwelling biliary stents and relation to potential factors involved in their occlusion.

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2 Peer-review report

Reviewer #1:

1. Abstract, and Materials & Methods sections. The quality of the biliary stent material, or producing company was not reported.

The quality of the biliary stent material and the producing company has been included in the results.

2. Results. Were the all biliary stents placed endoscopically?

Yes, all the biliary stents were placed endoscopically. This has now been mentioned in the results.

3. Results. Main data of the present study does not seem attractive.

Data presentation has been changed

Can the authors analyze when to replace the biliary stent to avoid stent occlusion or cholangitis based on their molecular analysis?

From our data, it seems that stents should be replaced between 3 and 6 months.

4. Discussion. The authors should elucidate something new to prevent biliary stent occlusion or cholangitis.

Our data suggests that between 3 and 6 months of stent placement, there is more biofilm formation, and hence more chances of stent occlusion /cholangitis. So stents should be replaced during this period.

Reviewer #2:

1. What's the concept of biofilms? Is it right to use the word "biofilms" for the biliary sludge in the occluded stent? If it is reasonable to use this concept of biofilms, please depict why?

Yes, it is reasonable to use the word “biofilms” which occlude the stents. Biliary sludge comprises of only mucus strands, cholesterol liquid crystals, monohydrate crystals and salts of calcium and bilirubin (Lee & Nicholls, 1986). Bacterial biofilms are formed when unicellular bacteria come together to form a community that is attached to a solid surface and get encased in an exopolymeric substance largely comprising of proteins and different extracellular polymers (Costerton et al, 1995). In the present study, numerous microorganisms were detected along with other biofilm constituents.

- (i) Lee SP, Nicholls JF., Nature and composition of biliary sludge. *Gastroenterology*. 1986 Mar;90(3):677-86.. PMID: 3943697
- (ii) Costerton JW1, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. *Annu Rev Microbiol*. 1995;49:711-45 PMID: 8561477

2. In fact, the authors detected the constituents from biliary stent retrieval, such as bacteria, protein and polysaccharide, which is not biofilms.

As mentioned above bacterial biofilms are formed when unicellular bacteria come together to form a community that is attached to a solid surface and get encased in an exopolymeric substance largely comprising of proteins and different extracellular polymers. This is different from sludge which comprises of only mucus strands, cholesterol liquid crystals, monohydrate crystals and salts of calcium and bilirubin.

3. As a foreign body, indwelling a biliary stent inevitably ensues future occlusion, the best way to avoid stent occlusion is early removal.

Yes, longer indwelling time is associated with biofilm formation. The risk of occlusion of standard polyethylene stents appears to increase progressively after 3 months (Donelli et al, 2007). So, stents should be removed between 3-6 months.

Reviewer #3:

1. What about the anthropometric measurements of the patients?

We do not have data on weight and height of patients.

2. How many patients were diabetics? It is important to consider in this group of patients to analyze the comorbidity index.

Comorbidities were not recorded. So we cannot answer this question. This has been added in limitations of the study.

3. I suggest analyze your results by gender and age group.

The needful has been done and data added. Thanks for the suggestion.

4. Conclusion section. I suggest rewrite this section. In its current form, it does not look as a conclusion.

As suggested conclusion has been rewritten