ORIGINAL RESEARCH

Effect of diode laser when used alone or in combination with various irrigants on root canal microbes-an in vivo study

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ABSTRACT

Objective: To evaluate the *in vivo* effect of diode laser when used alone or in combination with various irrigants on root canal microbes

Methodology: A total of 25 patients in age group of 15-45 of distal canal of Permanent molars were selected from patients who had visited to the Department of Conservative and Endodontic Dentistry, Chandra Dental College and Hospital, Barabanki. Healthy and cooperative patients were selected for this study. They were subsequently divided into five groups comprising of 5 patients each - In Group I, only diode laser was used. In Group II, diode laser was used in combination with 5% sodium hypochlorite solution. In Group III, diode laser was used in combination with 2% Chlorhexidine and in Group IV, diode laser was used in combination with saline whereas Group V was treated with a combination of Sodium hypochlorite, 2% Chlorhexidine and Saline solution. Before treatment and after treatment microbial assessment (pathogen, colony count and aerobe/anaerobe typing) was done. Data was analyzed using SPSS 21.0 software. Kruskal-Wallis and Wilcoxon signed rank tests were used.

Results and Conclusion: Positivity rate for aerobes ranged from 60% to 100% and for anaerobes 60% to 80% in study groups. *Staphyloccus aureus,* Coagulase negative *Staphylococcus, Streptococcus* and *Bacillus spp.* were the aerobic isolates. *Enterococcus faecalis* and *Pseudomonas intermedia* were the anaerobic isolates. Mean reduction in aerobic colony count ranged from 73.92±6.36% (Group I) to 83.88±10.85% (Group V). Mean reduction in aerobic colony count ranged from 70.43±24.08% (Group V) to 95.98±6.97% (Group II). Statistically there was no signfiicant difference among the groups. On evaluating the extent of reduction in microbial count, the only significant reduction was observed in Groups IV and V for anaerobes (p=0.048). This preliminary assessment showed that diode laser in combination with saline was comparable to a multimodal modality of NaOCl, 2%CHX and Saline combination.

Key Words: Diode laser, root canal disinfection, Chlorhexidine, Sodium hypochlorite, *in vivo*

INTRODUCTION

The outcome of root canal treatment of teeth depends on efficient disinfection of the root canal system and prevention of reinfection[i,ii]. However, conservative chemomechanical treatment for canal preparation and enlargement fails to provide complete removal of bacteria. The selective efficacy of different root canal disinfecting agents against different microrganisms is another barrier in the way to achieve complete disinfection of root canal. As of date, more than 300 species of microorganism are recognized as normal inhibitants of the oral cavity but only limited numbers of species have been isolated from infected root canals[iii]. To find out a disinfecting agent that is equally efficacious against all types of microorganisms is another big task which still remains unfulfilled. These difficulties in assuring full disinfection of root canal motivate the endodontists to look beyond the conventional root canal preparation and treatment methodologies and to look for newer and more effective solutions.

Light amplification by stimulated emission of radiation (LASER) is a new modality which seems to fill the gap between the conservative root canal treatment and the desired outcome by offering efficient penetration, versatile action against almost all types of microorganisms. Although, Lasers are being used in dentistry in general and endodontics in particular for a long time. The first laser use in endodontics has been reported by Weichman & Johnson (1971)[iv,v] who attempted to seal the apical foramen *in vitro* by means of a high power-infrared (CO_2) laser. However, the clinical application of lasers in endodontics started in the late 90s when the new delivery systems, including thin and flexible fibres and endodontic tips, were developed. Today, lasers can be used in various endodontic procedures such as: pulp capping/pulpotomy, cleaning and disinfecting the root canal system, obturation, endodontic retreatment, and apical surgery[vi].

In the recent years, Diode lasers, that emit radiation within the visible (mostly 660 nm) and infrared (810 to 980 nm) range of the electromagnetic spectrum have emerged as an alternative to chemomechanical disfinfection of root canal. As compared to Nd:YAG laser, diode lasers have higher absorption coefficient in water (0.68 cm^{-1}), diode lasers have lower penetration depth into the dentine (up to 750 µm)[vii], hence they can be considered to be safer and more effective for the purpose of root canal treatment. A number of *in vitro* studies have shown[viii,ix,x] have shown them to be effective in root canal treatment, however, there is dearth of *in vivo* studies enquiring this efficacy.

Hence, the present study was planned to carry out a clinical *in vivo* study to evaluate the role of laser with respect to its antimicrobial efficacy when used alone and in combination

with various traditionally used irrigants for reduction of root canal microbes. For this purpose, we have selected diode laser as the representative laser technique and have included 5% sodium hypochlorite, 2% Chlorhexidine and Normal saline as the representatives of different clinically used irrigants.

MATERIAL AND METHOD

The present *in-vivo* study was carried out in the Department of Conservative Dentistry and Endodontics of Chandra Dental College and Hospital, Safedabad, Barabanki (UP) in collaboration with RML Mehrotra Pathology Pvt. Ltd., Nirala Nagar, Lucknow after obtaining approval from the Institutional Ethics Committee.

For this purpose a total of 25 patients presenting with carious teeth with single root canal indicative of root canal treatment having spontaneous pain, pain on percussion and swelling and having single canal (type I morphology) indicated for root canal treatment were enrolled in the study. Patients with previously endodontically treated teeth, having clinical exposure to the affected tooth, having periodontal pocket more than 2mm deep and periapical sinus/fistula and having any generalized disorder or antibiotic therapy with in previous two months were excluded from the study. These patients were then randomized equally into five groups as follows:

Group I (n=5): In this group, only 810nm diode laser was used for the purpose of disinfection.

Group II (n=5): The root canal was irrigated with 5% sodium hypochlorite solution followed by 810nm Diode Laser.

Group III (n=5): The root canal was irrigated with 2% Chlorhexidine solution followed by 810nm Diode Laser.

Group IV (n=5): The root canal was irrigated with Normal saline followed by 810nm Diode Laser.

Group V (n=5): The root canal was irrigated with 5% sodium hypochlorite solution followed by normal saline, 2% Chlorhexidine solution and finally treated with 610nm Diode Laser.

Study Interventions

A total of 25 patients in age group of 15-45 of distal canal of Permanent molars were selected from patients who had visited to the Department of Conservative and Endodontic Dentistry, Chandra Dental College and Hospital, Barabanki. Healthy and cooperative patients were selected for this study. The procedure and its possible discomfort, risks and benefits were explained fully to the Patients and Guardians. Ethical clearance was obtained from ethical committee of Chandra Dental College and Hospital, Barabanki. The informed consent from Patients/Guardians was taken prior to investigation. The selected permanent teeth were randomly assigned and divided into 5 groups according to use of Root Canal instruments and following procedure were performed.

An IOPA X-ray of the involved tooth was taken and teeth showing periapical pathosis were included in the study. Teeth for the study included distal canal of lower third molar for simplicity of the procedure.

After the application of a rubber dam, the crowns were disinfected and dried; then the infected root canals were prepared mechanically using the step-back-technique up to a width of 0.5mm irrigating only with physiologic NaCl solution. A 25 no H-file was used for circumferental filing for 20 seconds to collect dentin chips from coronal & mid parts of the canal. Sterile 35 K-file was used for sampling from the apical part by reaming for 20 seconds. Sterile paper points were used to collect the transfer fluid and dentin chips. Sterile paper points and sampling H & K-files were placed into a test tube containing 10 ml of sterile saline. It was vortexed for 20 seconds. 50 microlitres of vortexed saline was applied to tryptic soya agar. The sample was then incubated at 37^{o}C for 48 hours. CFU/ml for each plate was calculated using a bacterial colony counter.

Subsequently, root canal treatment was carried out as per standard procedure. During root canal treatment, except for Group I, in all the other groups using designated irrigants. In Group I, distilled water was used

as irrigant. In Group IV, use of NaOCl, Normal saline and Chlorhexidine was done in cyclic order for a duration of one minute each.

This procedure was followed by the actual laser treatment. The root canals were irradiated with a 810 nm diode laser by Denlase, China. The laser output power ranges from 0.5–15 Watt. A pulse rate of 2–32 ms in pulsed mode and a frequency of 1.5Hz–250 Hz can be used. The laser can also be operated in continous wave (cw) mode. The target beam is generated by a He/Ne laser (533 nm, 1mW). For root canal treatment, we used a setting of 2 W at a pulse rate of 20 ms, 50 Hz.

The laser beam is transferred to the handpiece via a flexible glass fiber; in the handpiece it is coupled to the actual application-tip with an outer diameter of 300 µm. The handpiece and the application-tip were sterilized after each individual treatment. The optical fiber was inserted as far as the apex; the correct insertion depth was ensured by measuring. The laser was then activated and the root canal slowly irradiated from apical to coronal in continuous circling movements to treat all dentinal tubules. Irradiation was repreated 5 times at each laser treatment, each time for a period of 5 seconds with short breaks in-between.

Following irradiation, the tooth crown was cleaned with distilled water and the exposed root canal sealed with a sterile cotton pellet and zinc oxyphosphate cement. The post-treatment microbiologic samples were collected immediately after the procedure using the same protocol as described above.

Microbiological assessment of samples was done at Dr RML Mehrotra Pathology. Assessment of microbial strain and its load was done. Comparison of intragroup and within group change in microbiological profile was done.

Data Analysis: Data was analyzed using Statistical Package for Social Sciences (SPSS) version 21.0. Colony count has been shown as log of CFU/ml. Data has been represented in terms of mean, standard deviation and median values. A non-parametric evaluation plan was followed. Kruskal-Wallis test and Wilcoxon signed rank test was used to compare the data.

RESULTS

Out of a total of 30 patients enrolled in the study, a total of 5 (20%) each were allocated to groups I, II, III, IV, and V. In Group I, only diode laser was used. In Group II, diode laser was used in combination with 5% sodium hypochlorite solution. In Group III, diode laser was used in combination with 2% Chlorhexidine and in Group IV, diode laser was used in combination with saline whereas Group V was treated with a combination of Sodium hypochlorite, 2% Chlorhexidine and Saline solution (Table 1, Fig. 1).

All the patients were assessed for aerobic and anaerobic microbes. At baseline, for aerobes, all the five (100%) samples of Group IV were positive whereas 4/5 (80%) were positive in Groups II and III. For Groups I and III, positivity rate was 60%. Four aerobe pathogens were isolated – CONS, *S. aureus*, *Bacillus spp.* and *Streptococcus.* Statistically, there was no significant difference among groups with respect to positivity rate (p=0.384). For anaerobes, 3/5 (60%) of Group II were positive whereas in other groups 4/5 (80%) were positive. However, statistically, there was no significant difference in positivity rate (p=0.928). (Table 2, Fig. 2).

For aerobes, before treatment, mean colony count ranged from $21.60\pm37.38x10^3$ CFU/ml (Group II) to $24.80\pm41.44\ x10^3$ CFU/ml (Group I) in different groups. Median colony count ranged from 5 (Group III) to 19 (Group V). After treatment, mean aerobic colony count ranged from $2.60\pm3.78\ x10^3$ CFU/ml (Group III) to $6.00\pm9.67\ x10^3$ CFU/ml (Group I) in different groups. Median colony count ranged from 1 (Group III) to 4 (Group IV). After treatment, mean % reduction in aerobic colony count ranged from 73.92±6.36% (Group I) to 83.88±10.85% (Group V) in different groups. Median % reduction in colony count ranged from 76.53 (Group I) to 85.93% (Group III) (Table 3).

For anaerobes, before treatment, mean colony count ranged from 16.40±12.30 x10^3 CFU/ml (Group I) to 30.80±34.63 x10^3 CFU/ml (Group V) in different groups. Median colony count ranged from 7 (Group III) to 19 (Group V). After treatment, mean anaerobic colony count ranged from 1.40±3.13 x10^3 CFU/ml (Group II) to 13.60±16.89 x10^3 CFU/ml (Group V) in different groups. Median colony count ranged from 0 (Group II) to 9 (Group V). After treatment, mean % reduction in anaerobic colony count ranged from 66.25±26.15% (Group IV) to 95.98±6.97% (Group II) in different groups. Median % reduction in colony count ranged from 68.05 (Group V) to 100% (Group II) (Table 3).

On evaluating the data statistically, no significant difference among groups was observed either for pre-treatment or post-treatment aerobic and aerobic count or mean % reduction in aerobic and anaerobic count. However, on comparing the within group change, it was found to be significant statistically only for aerobes in Groups IV and V (Table 3).

DISCUSSION

Although, a number of *in-vitro* studies evaluating the antimicrobial efficacy of diode laser are available yet there are limited studies on their usefulness *in vivo.* Moreover, there are almost no studies evaluating the role of diode laser as adjunct to conventional chemomechanical disinfection methods. Hence, the present study was planned as an *in vivo* study to evaluate the usefulness of diode laser in disinfection of root canal when used alone or when used as an adjunct to normal saline and different chemical irrigants or their combination.

We took sodium hypochlorite (NaOCl) and Chlorhexidine (CHX), two of the most commonly used chemical irrigants and normal saline (NS) as the representatives and evaluated them independently as well as in combination with diode laser as adjunct and compared them against diode laser alone.

In several previous studies, diode laser has shown a high antimicrobial tendency when used in combination with chemical irrigants[xi].

In present study, both aerobic and anaerobic isolates were identified from root canals. Among aerobes, the isolated pathogens were Coagulase negative *Staphylococcus*, *Staphylococcus aureus, Streptococcus* and *Bacillus spp.* whereas among anaerobes, the isolated pathogens were – *E. faecalis* and *P. intermedia*. Compared to present study, Gajan *et al.* (2009)[xii] reported *Peptostreptococcus* as the most prevalent species followed by *Streptococcus, Porphyromonas,* and *Enterococcus faecalis*. They also found strains of *P. provetti, S. sanguis, S. salivarius, P. endodontalis*, and especially *E. faecalis* in the unsuccessfully-treated root canals. Although, researchers are of the view that root canal has a predominance of anaerobes[xiii], however, in present study, aerobes were isolated from 21/25 (84%) of samples whereas anaerobes were isolated from 19/25 (76%) of samples. However, one must not forget that primary root canal infection is a dynamic process and bacterial species dominating at different stages of this process differ. In experimental studies ter Steeg and van der Hoeven showed that the most important factors driving this process are: availability of nutrition, oxygen level (redox potential) and the local pH within the root canal[xiv]. Facultatively anaerobic bacteria often found in root canals in primary root canal infection grow well in anaerobiosis. However, their primary energy source is carbohydrates. Obviously that a decrease in availability of carbohydrates in the root canal occurs when there is no direct communication with the oral cavity. This fact limits growth opportunities for facultative anaerobes and could be responsible for a lower prevalence of anaerobes as observed in present study.

In present study, mean pre-treatment colony count for aerobes ranged from 21.60±37.38 x10^3 CFU/ml to 24.80±41.44 x10^3 CFU/ml (median range 5 to 19 x10^3 CFU/ml) in different groups whereas for anaerobes this count ranged from 16.40±12.30 x10^3 CFU/ml (Group I) to 30.80±34.63 x10^3 CFU/ml (Group V) in different groups. Median colony count ranged from 7 (Group III) to 19 (Group V). Following treatment, mean aerobic load ranged from 2.60±3.78 x10^3 CFU/ml to 6.00±9.67 x10^3 CFU/ml in different groups. Median colony count ranged from 1 to 4x10^3 CFU/ml in different groups whereas mean post-treatment anaerobic load ranged from 1.40±3.13 x10^3 CFU/ml to 13.60±16.89 x10^3 CFU/ml in different groups (Median colony count 0 to 9x10^3 CFU/ml). The study showed a % decline ranging from 73.92±6.32% to 83.88±10.85% in different groups for aerobic load and 70.43±24.08% to 95.98±6.97% in different groups for anaerobic load. Statistically, there was no significant difference among different study groups (Diode laser alone, Root canal treatment by NaOCl followed by Diode laser treatment, Root canal treatment by Chlorhexidine followed by Diode laser treatment, Root canal treatment by Normal saline followed by Diode laser treatment and Root canal treatment by cyclic alternation of NaOCl, normal saline and chlorhexidine followed by Diode laser treatment) in % reduction of either aerobic or anaerobic load. However, when within group change in pre- and post-treatment microbial load was evaluated statistically, the only significant change observed was for combination of normal saline followed by Diode laser treatment and for cyclic alternation of NaOCl, Normal saline and Chlorhexidine.

The findings in present study, thus suggested that use of Diode laser treatment either alone or following use of different chemical irrigants produced a similar effect and as such there was no additional benefit of adding chemical irrigant, however, combined use of multiple irrigants (NaOCl, Normal saline and Chlorhexidine) and combined use of normal saline followed by Diode laser treatment seemed to have a slight edge over the other treatment modalities, but it was evident for aerobes only.

In various *in vitro* experimental studies, diode laser has been shown to be highly effective[xi,1,2,3]. However, their adjunct use in *in vivo* studies has been reported rarely. In one such study, Garcez *et al.* (2008)[4] used a stagewise endodontic therapy and photodynamic therapy using two cycles of endodontic therapy followed by two cycle of PDT and showed a significant reduction in microbial load after each session of photodynamic therapy. In present study, we did not use such protocol and carried out only one session of endodontic therapy followed by diode laser treatment. In another *in vivo* study, Bonsor *et al.*[5] showed that though chemical irrigation resulted in sterilization of 86.7% of infected canals yet the number of sterile canals increased nearly by 10% following photodynamic therapy, thus indicating that augmentation of photodynamic therapy enhances the performance of chemomechanical disinfection. In present study, diode laser treatment was done in all the groups and it is difficult to state whether diode laser treatment homogenized the outcome of all the groups. One of the limitation of present study was lack of control groups for each combination. Owing to this being an *in vivo* study, we could not carry out assessment on subjects unexposed to diode laser therapy and thus found that as an adjunct, diode laser

surpasses the microbial efficacy of all the other endodontic treatment methods.

In view of the observations made in present study, one question that emerges is that whether chemical irrigants are essential at all? More so when we have a plan for laser therapy following root canal treatment. The results of present study show that diode laser therapy alone without chemical irrigation also performed with equal efficiency as obtained for its combination with different chemical irrigants and normal saline. Radaelli *et al.* (2003)[3] in an *in vitro* study also showed that irrespective of the type of chemical irrigant used, diode laser treatment provided a significant reduction in microbial load, thus indicating that diode laser treatment augments the chemomechanical microbial load reduction. One of the problems in different empirical studies is great variability in design and difference in type of chemical irrigants chosen. In present study, we used NaOCl, Chlorhexidine and normal saline. In a previous *in vitro* study Souza *et al.* (2010)[xv] found that diode laser treatment did not enhance the disinfection after chemomechanical preparation using NaOCl as irrigant. In present study, we are not in a position to comment over this aspect as we did post-treatment sampling only once, *i.e.* after the treatment with diode laser was over. The

reason for doing so was that we were searching for the best combination of chemical irrigant with diode laser treatment, however, we found that diode laser treatment itself was so strong even when used alone. Similar to results of present study, Preethee *et al.* (2012)[xvi] showed that diode laser irradiation in conjunction with conventional chemomechanical techniques showed a significant and similar elimination of *E. faecalis* in the apical third of root dentin. In their study, complete elimination of *E. faecalis* was observe din all the groups where Diode laser irradiation was done, thus indicating that type of chemical irrigant plays a little role in affecting the relative efficacy of different chemical irrigants used for root-canal treatment.

The extent of reduction in microbial load as observed in present study for diode laser treatment alone group for aerobes (73.92%) and anaerobes (85.80%) was slightly lesser than that reported by Gunwal and Shenoi (2013)[xvii] in their *in vivo* study who reported an overall reduction of 90% which was significantly higher than the chemical irrigant treated groups. Prabhakar *et al.* (2013)[xviii] in their *in vivo* study also showed a superior outcome of PDT when compared to NaOCl alone. However, the present study differed from these studies[xvii,xviii] as present study had all the groups where diode laser irradiation was done in all the groups.

CONCLUSION

The findings of present study, in general, support the view that diode laser therapy alone is sufficient to provide disinfection and prior chemical irrigation does not add value to disinfection. However, the results of present study might be viewed cautiously, owing to limitation of sample size, absence of control group where laser has not been used and *in vivo* nature of study, hence to corroborate the findings of study, further studies on larger sample size with adequate provision for control group (without laser use) are recommended.

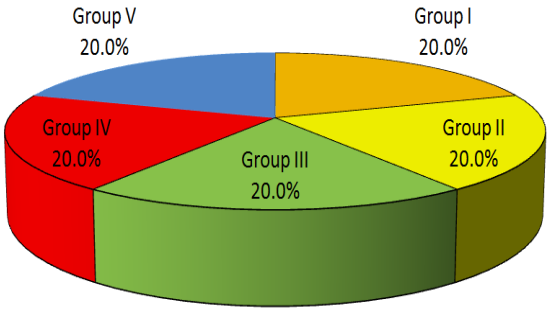
Table 1: Group wise distribution of cases

SN	Group	Description	No. of cases	Percentage
1.	I	Laser alone	5	16.7
2.	II	Laser + 5% Sodium Hypochlorite	5	16.7
3.	III	Laser + 2% Chlorhexidine	5	16.7
4.	IV	Laser + Saline	5	16.7
5.	V	5% Sodium hypochlorite + 2% Chlorhexidine + Saline	5	16.7

Group	Total No.	Aerobes			Anaerobes		
		No. positive	% Positive	Organisms	No. positive	% Positive	Organisms
I	5	3	60%	*CoNS, S. aureus*	4	80%	*E. faecalis, P. intermedia*
II	5	4	80%	*S. aureus, Bacillus spp.*	3	60%	*E. faecalis,*
III	5	4	80%	*S. aureus*	4	80%	*E. faecalis,*
IV	5	5	100%	*CoNS, S. aureus*	4	80%	*P. intermedia,*
VI	5	5	100%	*Streptococcus, S. aureus*	4	80%	*E. faecalis, P. intermedia*
Statistical significance		χ^2=4.17; p=0.384			χ^2=0.877; p=0.928		

Table 3: Table showing mean pre-and post-treatment Microbial count and % Reduction in microbial count in different groups

SN	Group	Pre Treatment Mean±SD (Median)	Pre Treatment Mean±SD (Median)	% Reduction	Significance of within group change (Wilcoxon signed ran test) 'p' value
(Aerobes in CFU/ml)x10^3					
1.	I (n=5)	24.80±41.44 (12.00)	6.00±9.67 (3.00)	73.92±6.36 (76.53)	0.109
2.	II (n=5)	23.20±18.13 (18.00)	4.00±3.87 (3.00)	81.58±15.01 (85.93)	0.068
3.	III (n=5)	21.60±37.38 (5.00)	2.60±3.78 (1.00)	82.86±14.89 (82.39)	0.068
4.	IV (n=5)	22.40±19.40 (18.00)	5.00±5.29 (4.00)	80.74±12.47 (77.78)	0.043
5.	V (n=5)	23.40±19.82 (19.00)	4.40±4.62 (3.00)	83.88±10.85 (78.95)	0.043
'p' value (Kruskal-Wallis test)		0.659	0.875	0.811	
(Anaerobes in CFU/ml)x10^3					
1.	I (n=5)	16.40±12.30 (16.00)	2.40±2.30 (3.00)	85.80±11.79 (84.09)	0.068
2.	II (n=5)	16.80±24.30 (7.00)	1.40±3.13 (0.00)	95.98±6.97 (100.00)	0.109
3.	III (n=5)	18.00±19.34 (11.00)	2.80±3.70 (2.00)	88.04±9.16 (86.07)	0.068
4.	IV (n=5)	19.20±17.63 (18.00)	2.40±2.30 (3.00)	88.28±11.71 (90.44)	0.068
5.	V (n=5)	30.80±34.63 (19.00)	13.60±16.89 (4.00)	70.43±24.08 (68.05)	0.068
'p' value (Kruskal-Wallis test)		0.961	0.706	0.588	



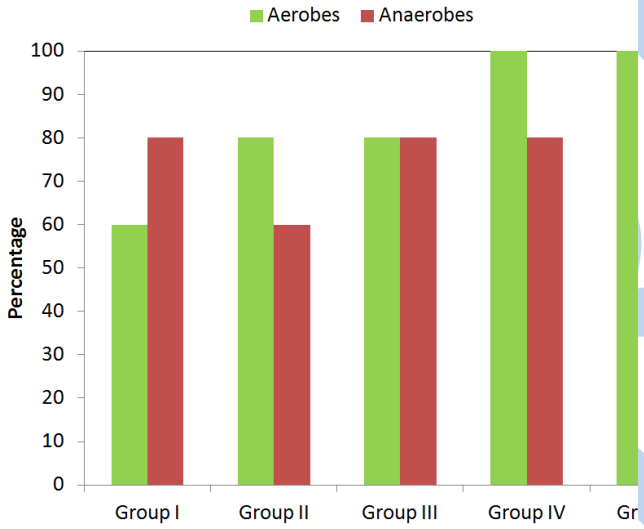


Fig. 2: Distribution of patients according to presence of aerobes and anaerobes

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