MULTIDOSE BEVACIZUMAB (AVASTIN) VIAL MICROBIAL SAFETY

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• Introduction

- Bevacizumab (Avastin) is a recombinant monoclonal antibody approved by FDA (Food and Drug Administration) for the intravenous treatment of colorectal cancer.[1]
- Its off-label intravitreal use has shown promise for the treatment of neovascular age-related macular degeneration, Proliferative diabetic retinopathy and iris neovascularization.[2-4]
- Bevacizumab is available in a vial containing 100mg in 4ml solution and one needs only 0.05ml for intravitreal injection that accounts to 1.25mg which is commonly used in an ophthalmology practice. Therefore, the contents of the vial can be used on multiple patients and there is significant cost saving .

Disclosure statement:

There are no financial conflicts of intrest.

- This study is carried out to know the microbial safety of Avastin Vials (with Multipuncture multi-dose utility) which were stored in refrigeration and re-used for 1 month each.

• Methods

- A Prospective interventional, single centre study was conducted over a period of 15 months (July 2019 till Sep 2020). Patients requiring intravitreal Avastin injections for various ocular conditions as Diabetic Macular Edema, Macular Edema secondary to venous occlusion, Choroidal Neovascular Membrane (CNVM) were included in this study after obtaining informed consent.

- A total of 12 sequential vials of bevacizumab (Avastin) were used for this study.

- First vial was opened in July 2019 (1st study month). Each time the vial was punctured with 26G needle, the rubber cap of the vial was wiped by Isopropyl alcohol, allowed to dry and disinfected with 10 % Povidone iodine.

- Prior utilizing each vial for intravitreal injections, bevacizumab drug from Avastin vial was drawn into 1CC with aseptic precautions and sent immediately to microbiology laboratory for bacterial and fungal cultures, Grams staining and KOH mount.

- All the vials were always returned to refrigerator and stored at 4^{o} C at procurement, in between the procedures as well as period after utility till 13th month study period. All the intravitreal injections were given in operation room under aseptic precautions using 30 G needle by a single ophthalmic surgeon. All patients receiving injections from these vials were clinically followed up till 15th study month end for clinical evidence of ocular infection or inflammation.

- **Sterility analysis:**

- Grams staining, KOH mount as well as Blood agar, MacConkey agar & Brain Heart Infusion Agar were used to assess microbial growth.
- MacConkey agar is used mainly in identifying lactose fermenting and gram-negative enteric pathogens and for inhibiting gram positive organisms.
- Blood agar is an enriched medium used to isolate fastidious organisms and detect hemolytic activity.
- Grams staining is one of the differential stains that are used to characterize bacteria in one of two groups: either gram-positive or gram-negative bacteria.
- Potassium hydroxide (KOH) preparation was used for detection of fungal elements in clinical specimen. Brain Heart Infusion agar was used for detection of fungal growth.

- **Results:**
- 12 sequential Avastin vials with in expiry period were included in this study. All vials were negative for bacterial and fungal growth at both evaluations.

- **Discussion:**

- Bevacizumab is preservative free anti-angiogenic drug which is intended for single use and the manufacturer recommends that each vial should be used within 8hrs after initial puncturing of the vial.

- A study has shown that refrigerated bevacizumab can be stored for up to 3wks at 4^{o}C without loss of efficacy.[6] The current practice related to storage of bevacizumab vials is to aliquot and store in 1ml plastic syringes for intravitreal injections whenever needed which is best done by compounding pharmacy.

- But such facility is not available in all ophthalmic set ups. Many other alternatives are being used which include either

1)Pooling the patients in a given day and usage of single vial of bevacizumab by puncturing multiple times.

2)Aliquoting without using the standards of compounding pharmacy.

3)Using single vial of bevacizumab until the drug in it gets completely exhausted.

- Pooling of patients may not be cost-effective because of wastage of the drug in low-volume centres. In addition, it is difficult to schedule the patients and also affects convenience of patients.
- By aliquoting the drug there is reduction of cost. The patient can be injected whenever required by opening one aliquot containing the required dose.
- However, Yannuzzi et al.[13]in his study showed that compounded bevacizumab, while being sterile, showed a significant variation in protein concentration.
- Intravitreal Avastin prepared by compounding pharmacy and injecting in remote areas, showed evidence of intra ocular infections in various reports
- With compounding, because of admitting into different manufacturing process and extra drug manipulation, use of Avastin may cause increased incidence of endophthalmitis after injection.[15]

- A study suggested that if aseptic precautions are followed, the contents of multiple-dose vials stored at 4 degrees Celsius will remain sterile and the anti-VEGF activity of bevacizumab stored at 4 degrees Celsius will remain stable for up to 6 months.[8]

- Drawing multiple doses from the same vial immediately before injection has the potential for contamination. Increasing the number of punctures also increases the risk of contamination.

- This could be due to the rubber septum increasingly wiped by fingers or a dirty gauze, rubber stopper leakage, poor aseptic technique (eg. entering the vial without alcohol swabbing), injection of air into the vial before removal of the solution, a contaminated needle or syringe used to draw medication, and inappropriate storage durations and temperatures.[9-11]

- Our study showed that if proper aseptic precautions are taken like cleaning the rubber cap with Isopropyl alcohol and disinfecting with 10 percent Povidone iodine along with maintaining proper storage conditions and negative pre-injection cultures, multi-use vial retains sterility over at least a year's time for its extended use.

- Chen et al.[12] showed that if proper aseptic precautions are taken while using bevacizumab, the contents of the multidose vial stored at 4°C for 6 months will remain sterile, even with repeated exposure to room temperature during withdrawal.

- The authors checked for sterility at 0, 1, 3, and 6 months. In our study we checked sterility of the contents of multidose vial over a period of 1 year which doesn’t show any microbial growth.

- Kamath et al.[17]demonstrated that by maintaining proper storage and usage precautions Bevacizumab vial can be used for multiple times.This study was done over a period of 20 months and the vial was used for 1 month which was discarded later regardless of volume.

- However, surgeons should be aware of legal issues regarding the use of preservative-free single use Bevacizumab vial for administrating multiple doses.

- None of the patients who received injection from such vials showed clinical evidence of infection or inflammation on follow up which consolidates faith in use of multidose Avastin vial for intravitreal injections

- **Conclusions:**

- In this study, the contents of multiple-dose Bevacizumab (Avastin) vials remain sterile for a period of one year.
- Multi puncture, multi-dose Avastin vial utilization with due aseptic precautions and storage shows reliable microbiological safety both in vitro and in vivo over 1 year period.

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THANK YOU