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ASSESSMENT THE MICROBIAL CONTAMINATION RATE OF RE-USED VIALS OF BEVACIZUMAB (AVASTIN) AND THE RISK OF ENDOPHTHALMITIS.

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Abstract

Background: Introduction of anti-VEGF therapy has changed the way of management to the retinal diseases. Pegaptanib (Macugen)was the first to be approved and was followed by other agents, including ranibizumab (Lucentis, Genentech, San Francisco, Calif.), bevacizumab (Avastin, Genentech) and aflibercept (Eylea, Regeneron, Tarrytown, N.Y.).
Studies have shown the safety and efficacy of these agents in the treatment of wet Age Related Macular Degeneration(ARMD), Retinal Vein Occlusion (RVO)and Diabetic Macular Oedema(DME). These drugs delivered into the vitreous cavity through pars plana injection, and are generally safe with little serious complications.
Aim: To evaluate the rate of microbial contamination and safety aspects of multi puncture vials of bevacizumab (Avastin) and to determine the rate of endophthalmitis .
Patients and Methods: A multi-centric prospective study conducted at the Basrah general teaching hospital and Al-Zubair general hospital, Basrah, Iraq, a total of 28 consecutive vials of bevacizumab {Avastin; F. Hoffmann-LaRoche Ltd. Switzerland} were used. A total 1096 injections were administered to 899 patients between September 2019 and November 2024. After puncture the vial, multidose used for intravitreal injections, residue bevacizumab in the vial was stored in the refrigerator at 2-8℃ and reused for up to 4 weeks. From each vial, a dose of 0.05 ml was withdrawn to a sterile 1ml syringe under aseptic technique and sent for bacterial gram staining and culture.
Results: No evidence of microbial contamination was detected in 28 vials as approved by the results of microbial staining and culture. One patient of 899 patients who received intravitreal injections from these vials showed signs of inflammation, otherwise; no evidence of endophthalmits.
Conclusions: This study propose that the content of multipuncture bevacizumab (Avastin) vials remain sterile over a period of 4 weeks without any microbial contamination if perfect aseptic and storage precautions are maintained. Using multidose of bevacizumab vials for intravitreal injection which stored for up to 4 weeks in refrigerator at temperature of 2-8 ℃ is safe.
Keywords: Bevacizumab, Endophthalmitis, Microbial contamination, Multi-use vials.

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Introduction

Studies have shown the safety and efficacy of these agents in the treatment of wet ARMD, RVO and DME. These drugs delivered into the vitreous cavity through pars plana injection, and are generally safe with little serious complications. [1,2]

Bevacizumab is full length humanized VEGF inhibitor synthetized by recombinant genetic engineering from the same murine monoclonal Antibody against VEGF angiogenic protein thought to play an essential role in the formation of choroidal neovascular membrane. Bevacizumab is therapeutically indicated mainly for treatment of neoplasms, such as metastatic colorectal carcinoma, lung cancer, breast cancer, metastatic renal cell cancer and glioblastoma. Its off-label drug in ophthalmology by FDA .The Bevacizumab is available in 100mg/4ml and 400mg/16ml vials and preservative free , hence, manufacturer recommends the use of the vial for single use and that the vial discarded after 8 hours of the initial puncture of the vial ,However, small fractionated doses withdrawn (1.25mg/0.05ml) to be ready for intravitreal injection,this dose fractioning make the cost is low as compared to the other FDA approved intravitreal AntiVEGF,However the risk of contamination and endophthalmitis is not

Vascular endothelial growth factor is one of the several proangiogenic cytokines that is overexpressed in the vitreous cavity in variety of retinal vascular diseases as well as other vasoformative retinal diseases.

This overexpression leads to vascular endothelial cell proliferation and the formation of abnormal blood vessels in the the retinal as well as subretinal space. This is postulated to be the causative in the underlying pathology of various ocular diseases, including diabetic retinopathy (DR) specially in advanced proliferative DR, it reaches to 3- folds of the normal and, neovascular age related macular degeneration and retinal vein occlusion. This abnormally fragile blood vessels may have crucial role in visual loss secondary to oedema, hemorrhage, and/or fibrovascular proliferation and subsequently, retinal detachment.

Introduction of anti-VEGF therapy has change the way of management to these retinal diseases. Pegaptanib (Macugen)was the first to be approved and was followed by other agents, including ranibizumab (Lucentis, Genentech, San Francisco, Calif.), bevacizumab (Avastin, Genentech) and aflibercept (Eylea, Regeneron, Tarrytown, N.Y.)

survey despite the dreaded risk of visual loss from cluster endophthalmitis. [13,14,15] Another point with regard the wide use of the off-label use of bevacizumab is the apparent similarity in the therapeutic efficacy with the FDA-approved Anti-VEGF the ranibizumab (CATT study).[16] In this study, we use bevacizumab stored into aliquot single-use 1mm plastic syringes and/or vials.

Method:

A prospective observational, multi-centric study was conducted at Basra Teaching Hospital and AL- Zubair General Hospital from September 2019 to November 2024. The study obtained the approval from the Ethics Committee of the hospital and the Department of Scientific Research and Development in the General Health Directorate of Basrah, a 28 vials of bevacizumab were evaluated and used in period between September 2019 and November 2024,and each vial used for 4 weeks .The vials were stored at refrigerator at 2-8 ℃ after opening .The vials were inspected for any turbidity of the content and/or any changes in color before each use. In case. were they detected to be turbid or changed its color content, they were discarded on the presumption that they could possibly be contaminated. We included vials that were not beyond their expiry date and had been used to inject negligible. [3-6] Single bevacizumab vial can be used to inject many patients on the same day (pooling of the patients) and several days and weeks provided it is well stored in refrigerator at temperature of 2-8 ℃ and at the same time recent reports demonstrated that this refrigerated bevacizumzb for up to 4 weeks were safe and effective, supported by different strategies to evaluate the sterility of single multi pierced vials.[7,8]

Despite its off-label use in ophthalmology [9-12], bevacizumab still widely used in different parts of the world, specially, the developing countries, where the demand for intravitreal Anti-VEGF is great issues in treatment a lot of retinal vascular diseases, of great concern is diabetic retinopathy.

Those patients have a lot of suffering to offer the cost of frequent Anti-VEGF, hence, this burden the health care system in these countries. In an era of increasing health care costs and quality-control mandates, compounding pharmacies have been under intense scrutiny. The health care system, including ophthalmologist, rely on compounded drug formulations particularly intravitreal bevacizumab. Substantial cost savings may be derived from using bevacizumab.

In india the use of off-label and multi dose bevacizumab vial is increasingly prevalent procedure, as evident from indian surgeons

needle, then fractionated dose of 0.05ml of Avastin withdrawn by 0.1ml syringe and injected intravitrealy with 30G needle 3.5-4 mm from the limbus, after injection sterile ophthalmic sponge soaked with 5% povidone iodine applied at the site of injection for 2-3 minute to prevent reflux and vitreous leak.

Postoperative topical antibiotic eye drops (gatifloxacin eye drop)prescribed four times daily for 7 days. These patients were examined after 1 day,7days, 30 days and 42 days for any signs and symptoms of inflammation and/or endophthalmitis such as decreased vision, pain, floater, eyelid oedema, hypopyon, corneal edema and vitritis. After injection of all patients scheduled on that day, the 26G needle was removed from the vials and the vial rubber bung ringsed with antiseptic solution and subsequently stored in clean refrigerator at 2-8c to be used again.

candidate patient for various ocular conditions as diabetic macular edema, macular edema secondary to venous occlusion, and choroidal neovascular membrane at predetermined date. From each vial and after each re-use. Two samples of 0.05ml of Avastin withdrawn with 1 ml syringe at 1,2,3 and 4 weeks after the opening the vial. Before and after each use, the vials were sprayed and soaked with 70% ethyl alcohol and anti-septic solution, the patients received informed consent and detailed explanation about the risk and possible complications of the procedure. Under aseptic technique which include rinsing repeatedly the conjunctival sac, fornices, eyelid margin and lashes with povidone-iodine 5% and topical anesthesia with tetracaine eye drop at least three times at 5-10 minutes interval. The procedure was done in the operating theatre. The Avastin vial punctured by sterile 26G

Result:

We studied the endophthalmitis risk on the eyes of 899 patients whom they received intravitreal(0.05-0.1) ml of Avastin, withdrawn from re-used Avastin vial. Before each injection, a sample of Avastin were sent for culture and bacterial gram staining . No bacterial growth was documented during the whole period of study. Of 899 patients were 430 male and 579 were female. Total number of patients who received intravitreal injection from the initial opening of the bevacizumab (Avastin) vial were 393(group 1) , whereas those who received intravitreal injection from multipuncture vials 616 patients (group 2).Majority of patients were elderly in age group (50-70 years), female more than males and majority having diabetic retinopathy (DR) 80%, followed by retinal venous

occlusive diseases 10.6%,age related macular degeneration(ARMD) 4.33% as demonstrated in table (I) and table(II).

Table I: show age, sex and retinal pathology for group (1).

Demographics of patients who received intravitreal injection from the 1st opening of the vial		
Age	No. of patients	%
20-29	25	6.36%
30-39	34	8.6%
40-49	59	15%
50-59	137	34.86%
60-69	78	19.84%
70-79	42	10.68%
80-89	39	9.9%
Total	393	100%
Sex		
Male	149	37.9%
female	244	62.1%
Total	393	100%
Retinal pathology		
DR	334	84.98%
Retinal vein occlusion	31	7.8%
ARMD	15	3.8%
others	13	3.3%
Total	393	100%

(NB: DR. Diabetic Retinopathy, ARMD. Age Related Macular Degeneration.

Table II: demonstrate the age, sex and retinal pathology for candidate patients for weeks after opening the vials.

Demographics of patients received intravitreal bevacizumab from multipuncture vials					
Variable	No. of patients	Duration after opening			
Age		1st week	2ndweek	3rdweek	4thweek
20-29	30	15	10	5	0
30-39	42	20	12	6	2
40-49	90	65	20	5	0
50-59	140	80	40	13	7
60-69	136	96	28	9	4
70-79	52	40	10	2	0
80-89	15	9	3	2	1
Total	506	325	123	42	14
Sex					
Male	241	163	46	38	8
Female	265	162	77	4	6
Total	506	325	123	42	14
Retinal pathology					
DR					
Retinal vein occlusion	391	308	59	18	8
ARMD	65	15	33	15	2
Others	24	0	17	7	0
	26	2	14	2	4
Total	506	325	123	42	14

In the table (3), the number of intravitreal injections received by the patients were more in the 1st week and gradually decreased over the period of 4 weeks. No bacterial growth detected from the withdrawn sample pre and post injection, and similarly no infectious endophthalmitis observed during the follow up period.

Table III: Number of intravitreal injections received in the 1st,2nd ,3rd and 4th weeks with the microbial results pre and post injection and the presence of endophthalmitis.

Time elapse from the 1st opening of the vial (week)	No. of injections	Microbial assessment (Gram staining, blood Agar culture)		Presence of Endophthalmitis Yes/No
		Preinjection	postinjection	
1st Week	450	Negative	Negative	No
2nd Week	164	Negative	Negative	No
3rd Week	70	Negative	Negative	No
4th Week	17	Negative	Negative	No

severe ocular or systemic adverse effects (such as retinal detachment, vitreous hemorrhage, cataract, hypertension, heart failure, or thromboembolic phenomena).

Discussion:

Infectious endophthalmitis is potentialy devastating complication after any intr-ocular procedure and its of great concern to the ophthalmologist who perform these procedures .[17]

Vascular endothelial growth factor inhibitors have shown to be revolutionized the treatment of Diabetic Retinopathy,Retinal vein occlusion and Age Related Macular Degeneration

Those patients were examined regularly at follow –up visits after 1 day,7 day, 30 days and 42 days for any signs and symptoms of inflammation or endophthalmitis such as pain, decreased vision, floater, conjunctival congestion, eyelid oedema, corneal oedema, hypopyon, vitrits, vitreous hemorrhage were observed closely.

None of the 899 patients had clinical picture of endophthalmitis during the follow- up period, only one patient developed sterile endophthalmitis proved by negative vitreal sample for bacterial culture and its resolution by systemic steroid. No other identifiable significant

cost approximately $2 billion or one-sixth of the entire Medicare Part B drug budget. In2013, Medicare Part B expenditures for aflibercept and ranibizumab alone $2.5 billion .From a social perspective ,bevacizumab as first- line therapy for Diabetic Macular Oedema(DME) would confer the greatest value,along with substantial cost saving vs. other agents (Adam RGlassman,of thejaeb centre for Health Research, Tampa,Fla,and colleagues examined the incremental cost–effectiveness ratio of aflibercept ,bevacizumab and ranibizumab for the treatment of DME with an analysis of efficacy ,safety and resource utilization data at one year follow up from the Diabetic Retinopathy Clinical Research Network Comparative Effectiveness Trial)

Patient can receive the intravitreal Avastin when indicated from already prepared sterile fractionated dose. There are several studies established the stability and efficacy of stored Avastin vials for a period of 6 months and also that a single vial can be safely used for up to 10 consecutive injections as far as sterile practices are perfectly undertaken.[1]

Howover,in this study stability of stored bevacizumab not involved.The incidence of endophthalmitis in this study is comparable to what recorded in literature

Bevacizumab is cheaper than other Anti-VEGF like ranibizumab,pegaptanib and afliberecept.Nevertheless,it shows the same efficacy of ranibizumab according to CATT study.Bevacizumab available in vial of 100mg/4ml and 400mg/16 ml,accordingly it is possible to inject tens of eyes from each vial taking in consideration the wastage amount during fractionation and injection.Its stated that each vial is intended for single use only and that injection better to be used within 8 hours after piercing the vial.Hence,the main drawback of concern is the development of post injection endophthalmitis.

The advantage of using multi-pierced bevacizumab vials is the reduction of cost, in many developing countries where Diabetic Retinopathy is sight crippling problem patients cannot offer the cost of the drug specially that those individuals need more than one injection and probably for a period of more than one year, the cost of Avastin injection approximately 600$ considering that the patients received nine to 11 times in the first year of treatment ,17 times during five years, total costs can be substantial, In 2010, when these drugs were being used for Age –related macular degeneration ,ophthalmologic use of Anti VEGF therapy

In this study,none of the samples reveals microbial grouth during the the incubation peroids.Prolonged storage in refrigerator,handling during preparation,and keeping the syringes together,whether in sterile or non sterile container, did not result in contamination of the content.The proposed storage time of bevacizumab is 24 months when sored at 2-8c,45 days at 3-5c and up to5 freeze/thaw cycles.It has been demonstrated that bevacizumab can be withdrawn into a syringe and stored for up to 6 months with minimal degradation.The product has been shown to be sterile with preserved efficacy when stored at 4c for up to 3 weeks.[18-21]

Based on the short term sterility of our study,extracting multiple doses from the vials and storing them in refrigerator in various ways seems to be safe.The use of commercially available prefilled syringes is the best safe way to avoid vial contamination.To be safely used multiple doses of bevacizumab from the original stored vial after withdrawling with sterile needle ,its better to decontaminate the vial stopper with 70% alcohol each time ,no doubt that the legal issues pertaining to the use of preservative_free single_use vial for delivering multiple doses be available.[22,23]

irrespective of method of preparatoion of IVB injections,Despite a septic precautions such as wiping the cap of the vial with alcohol swab and /or betadine before each aspiration from the bavacizumabvial directiy,still there is risk of contamination of the rubber cap of the vial by multiple punctures.If contamination of the vial occur during multipuncture and stored for longer period of time,the bacterial load within the vial may gradually increase,this augmented by the abscenc of any preservatives in the vial that may inhibit the growth of bacteria.However,for economic reasons,the use of bevacizumab for intravitreal use still going on.Hence,strict measures should be taken to mitigate the risk of endophthalmitis.[6]

The intravitreal injection of anti-VEGFs is becoming one of the most widely used ophthalmic procedures due to the good visual results in various retinal diseases. the sterile technique of preparing and injection the drug with encouraging result of absence of endophthalmitis has to be taken in consideration.

Drug sterility can be maintained in these small syringes or in the original vials, such way giving support to the strategy of multi used avastin vials stored at refrigerator, then significant cost savings, with product conservation, would be achieved.

Conclusion

Based on the results of our study, we conclude that use of fractionated doses of bevacizumab from re-used vial stored in refrigerator at 2-8c and up to 4 weeks is a safe. Using the same vial several times and fractionated small intravitreal doses don't increase the chance of endophthalmitis. This can be applied in occasions where there was shortage in the supply of the Anti VEGF.

In this study ,loss of patients adherence is challenging during the period of covid -19 lockdown ,whether stored fractionated bevacizumab effective or not need further study ,we don't assess the sterility and safety of the first opening of the vial at 1st day of opening since the study was primarily designed to evaluate the safety of of using multi –puncture bevacizumab stored in refrigerator at 2-8c,therefore,assessing the sterility and safety of new avastin vial not included in this study.

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Authors' Contributions:

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Work concept and design 1,2
Data collection and analysis 2,
Responsibility for statistical analysis 2
Writing the article 1,2
Critical review, 1, 2
Final approval of the article 1,2

Each author believes that the manuscript represents honest work and certifies that the article is original, is not under consideration by any other journal, and has not been previously published.

Availability of Data and Material: The corresponding author is prompt to supply datasets generated during and/or analyzed during the current study on wise request.

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