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Original article

Chlorhexidine alcohol versus povidone-iodine: The comparative study of skin disinfectants at the blood transfusion centers of Iran

Alcool chlorhexidine versus povidone-iode : étude comparative des désinfectants cutanés dans les centres de transfusion sanguine d'Iran

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ABSTRACT

Objectives. – The skin disinfection in the blood donor's arm is a key step to minimize the risk of microbial contamination at blood donation sessions. Current study aimed to compare the efficacy of 2% chlorhexidine gluconate in 70% isopropyl alcohol (CHG/IPA) with povidone-Iodine (PI) at blood transfusion centers (BTCs) of Iran.
Material and methods. – Blood donors were selected to evaluate three commercial CHG/IPA disinfectants ($N = 300$), prior the application at BTCs, and to compare the rate of positive skin cultures between CHG/IPA and PI in 31 BTCs ($N = 8578$). The rate of positivity for PI over a 5-year period was also investigated. After application of a two-step disinfection procedure, the biochemical characteristics were checked in accordance with the conventional bacteriological methods. The Z-test analysis was used to compare the deviation between the positive microbial culture ratios.
Result. – No donors had a positive culture after disinfection during the evaluation study. There was no difference in the rate of positivity between PI and CHG/IPA after disinfection ($P > 0.05$). The rate of positivity for PI from 2012 to 2017 showed a decreasing trend. The rate of positivity was significantly higher in winter rather than summer ($P < 0.05$).
Conclusion. – The disinfection efficacy of CHG/IPA was equivalent to that of PI. The 5-year monitoring of PI at BTCs showed that the improvement in the rate of positive skin cultures possibly due to effectiveness of correcting actions.

Mots clés :
Chlorhexidine
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Donneurs de sang
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RÉSUMÉ

Objectifs. – La désinfection de la peau dans le bras du donneur de sang est une étape clé pour minimiser le risque de contamination microbienne lors des séances de don de sang. L'étude actuelle visait à comparer l'efficacité du gluconate de chlorhexidine à 2 % dans l'alcool isopropylique à 70 % (CHG/IPA) avec la povidone-iode (PI) dans les centres de transfusion sanguine (BTC) d'Iran.
Matériel et méthodes. – Les donneurs de sang ont été sélectionnés pour évaluer trois désinfectants commerciaux CHG/IPA ($n = 300$), avant leur utilisation aux CTB, et pour comparer le taux de cultures cutanées positives entre CHG/IPA et PI dans 31 CTB ($n = 8578$). Le taux de positivité de l'IP sur une période de 5 ans a également été étudié. Après application d'une procédure de désinfection en deux étapes, les caractéristiques biochimiques ont été vérifiées conformément aux méthodes bactériologiques conventionnelles. L'analyse du test Z a été utilisée pour comparer l'écart entre les rapports de culture microbienne positifs.

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Résultats. – Aucun donneur n'a eu une culture positive après désinfection pendant l'étude d'évaluation. Il n'y avait pas de différence dans le taux de positivité entre PI et CHG/IPA après désinfection ($p > 0,05$). Le taux de positivité pour PI de 2012 à 2017 a montré une tendance à la baisse. Le taux de positivité était significativement plus élevé en hiver plutôt qu'en été ($p < 0,05$).
Conclusion. – L'efficacité de désinfection de la CHG/IPA était équivalente à celle de l'IP. Le suivi quinquennal des IP aux CTB a montré que l'amélioration du taux de cultures cutanées positives peut-être due à l'efficacité de la correction Actions.

1. Introduction

It has been proposed that the majority of bacterial species found in contaminated blood components are members of the permanent or temporary microbial flora of human skin [1]. Such a contamination in the blood bag apparently occurs during the procedure of blood collection by the needles [2–5]. Some of these bacteria may have insignificance clinical consequences for the blood recipient, but some species can cause severe infections. In the most severe cases, these infections could result in high rates of morbidity and mortality [5,6]. Food and Drug Administration (FDA) has reported that microbial infections have been responsible for almost 8% of deaths caused by blood and blood components transfusion from 2008 to 2013 [7]. Therefore, appropriate disinfection of skin in the antecubital fossa of blood donors' arms is critical, and could be regarded as the most effective method to minimize the risk of microbial contamination during blood donation sessions [4,8–10].

A two-step procedure recommended for the arm disinfection involves an initial scrub with 0.7% povidone Iodine (PI) followed by a secondary disinfection with 10% PI [11]. However, chlorhexidine-based methods are also suggested for skin disinfection in clinical settings such as catheter insertion [1] and recommended for blood donors sensitive to iodine [11]. Chlorhexidine is a cationic bisbiguanide antiseptic [12] with bactericide or bacteriostatic properties against a wide variety of Gram-positive and Gram-negative bacteria [1,13]. This compound reverses the charge on the surface of bacteria and leads to leakage of cytoplasmic content, which is followed by bacterial death [13].

Plenty of blood centers around the world have evaluated antibacterial properties of chlorhexidine in isopropyl alcohol disinfection method before the implementation and mostly have reported the effectiveness of this antiseptic over PI [1,2,4,5,7,9,10,13–16]. However, some studies have reported a less antiseptic effect for chlorhexidine in isopropyl alcohol than PI [5,8], while there are report of donors allergic reactions [2]. Therefore, due to controversy in this area, a comprehensive study is still required.

Iranian blood transfusion centers (BTCs) use a two-stage application by 0.7% PI. PI was not available in prepacked single-use form in BTCs and the problem was to manually prepare a 0.7% solution from a 10% stock. Therefore, there was a risk of transmission of microbial contamination through the solution and also a general dissatisfaction of the staff. To address this issue, Iranian Blood Transfusion Organization (IBTO) changed from two-stage PI application to 2% chlorhexidine gluconate in 70% isopropyl alcohol (CHG/IPA) as ready-to-use solution. Application of the new method required an initial evaluation prior to implementation. In this study, three commercial CHG/IPA disinfectants were evaluated. To ensure the results of the study in a local setting, it was necessary to compare the efficacy of CHG/IPA with that of PI under standard conditions by assessing the rate of positive skin aerobic cultures at Iranian BTCs. In addition, five-year monitoring of skin aerobic cultures of PI was discussed and a seasonal variation on the frequency of positivity rate was analyzed.

2. Materials & methods

2.1. Evaluating the three commercial CHG/IPA disinfectants

Three commercial disinfectants as spray bottles, Epimax (Emad Pharma, Iran; Cat No. 1228124471), BodyPrep (Behban Chemistry, Iran; Cat No. 1228142901), and Germo@ (Germo S.P.A, Italy) were used for 300 male volunteer blood donors (both regular and first time) referred to Tehran BTC, Iran. The sample selection process was performed randomly. All donors completed an informed written consent in accordance with the standard protocol of IBTO.

2.2. Comparative evaluation of efficacy of new and old disinfection methods in routine operation

The results of donors' skin aerobic cultures performed by the quality control (QC) department of IBTO were used to compare the efficacy of CHG/IPA with the PI method under routine circumstances in 31 BTCs during the first and second six-month of 2017 for PI and CHG/IPA, respectively. Due to recent application of CHG/IPA method in BTCs, only the results of the second six-month of 2017 are mentioned in the current study.

Routinely, the sample sizes at each BTC were based on the number of donation per month. As well, a five-year investigation was conducted to evaluate the positive rate of skin aerobic cultures at BTCs (from 2012 to 2017) for the PI disinfection method. Considering the impact of winter and summer seasonal variations, the summer and winter results were analyzed separately.

2.3. Disinfection procedure

PI: a 30-second initial scrub with a sterile swab soaked in a solution of 0.7% PI followed by a 30-second secondary swab with 10% PI in a concentric spiral manner and a 30-second drying period. The 0.7% dilution was manually made from the 10% stock. This procedure was routinely used at BTCs.

CHG/IPA: an initial spray with CHG/IPA and a 30-second hand scrubbing with sterile gauze or swab in a concentric spiral manner followed by a secondary spray with CHG/IPA and a 30-seconds drying. This procedure started at BTCs after the evaluation.

2.4. Sampling and culturing

Sampling: sampling was carried out by a sterile cotton-tipped swab/applicator soaked in normal saline. Samples were obtained from non-venipuncture arms and were taken before and after disinfection for the evaluation study of three commercial CHG/IPA disinfectants. But, to monitor the effectiveness of new and old disinfection methods (both CHG/IPA and PI) in routine operation at BTCs,

all samples were taken after disinfection of donor's venipuncture arm.

Primary culture: the swab taken from the arm was directly placed in a culture tube containing thioglycolate medium and incubated for 7 days at 37 °C. During the incubation, the medium was daily examined for the presence or absence of turbidity/cloudiness.

Secondary culture: a re-culture of thioglycolate medium was performed on blood agar plates and incubated for 48 hours at 37 °C.

The biochemical characteristics were checked using Gram staining, motility, catalase, and oxidase tests according to the conventional bacteriological methods at IBTO QC department.

2.5. Controls

Positive control: to evaluate the provided culture media, *Staphylococcus aureus* (ATCC 25923) was used as a positive control. From the suspension, a loopful was cultured on blood agar and another loopful in thioglycolate medium, and the cultures were incubated at 37 °C.

Negative control: two negative control samples were used. A negative control of the blank culture medium (unopened plates) was considered to check the non-contamination of the medium. Another negative control, similar to routine sampling was used (without swabbing from the arms) to demonstrate the non-contamination of some parameters affecting the procedure such as the sterility of swab, disinfectant agent, and normal saline and the sampling environment for cross contamination.

2.6. Investigating the CHG/IPA reactions

In the current study, any allergic reactions and complaints from CHG/IPA disinfectants, both in blood donors and staff, during the evaluation were considered and recorded.

2.7. Statistical analysis

Two population proportions with independent samples between CHX/IPA and PI groups, and between two different periods were compared using the z-test analysis by SPSS software (IBM Corp, Version 21.0). *P*-values equal or less than 0.05 were regarded as significant.

3. Results

3.1. The evaluation of CHG/IPA disinfectants

The Table 1 illustrates the results of bacterial growth assay and the variation of microbial flora on the antecubital fossa prior to disinfection. *Staphylococcus epidermidis* had the highest frequency of microbial flora. After disinfection with each of the three CHG/IPA sprays, no donor had a positive culture. Regarding the same result for three disinfectants, IBTO used them at BTCs.

3.2. The efficacy comparison of CHG/IPA and PI methods

The Table 2 presents the results of skin aerobic cultures in the studied BTCs. The Z-Score of compared differences between the positive microbial culture ratios of PI (1.97%) and CHG/IPA (1.87%) during the first and second six-month periods in 2017 was 0.47. The *P*-value for this analysis was not significant ($P = 0.63$).

To eliminate the seasonal effects on the results, a comparison was made between the positive microbial culture ratios of CHG/IPA results (1.87%) in 2017 with that of the PI (2.39%) in the same period in 2016. The Z-Score of compared differences was 2.10 with a significant *P*-value lower than 0.05 ($P = 0.03$).

As well, the Z-Score of compared differences between the positive microbial culture ratios of PI during the first and second six-month periods from 2012 to 2016 was 1.37. The *P*-value was not regarded as statistically significant ($P = 0.16$).

As demonstrated by Table 2, there was a decreasing trend in the overall rate of positive skin cultures from 2012 to 2017 at BTCs, while there was an increasing trend in the sample size for skin cultures.

According to the data presented in Table 3, the Z-Score of compared differences between the positive microbial culture ratios in summer and winter from 2013 to 2017 (including the CHG/IPA results) was 4.37 and the results were significant with a *P*-value equal to 0.007.

3.3. Controls

No bacterial growth was detected on the blood agar plates and thioglycolate medium used for the blank medium, swab, and disinfectant controls. *S. aureus* was detected in blood agar/thioglycolate medium as a positive control. However, over the years 2012 to 2016, there were rare reports from some BTCs QC departments for the positivity of negative control of the procedure, which was mainly related to the PI solution making.

3.4. Investigating the reactions caused by CHG/IPA

The allergy and complaints reported from both blood donors and staff at three BTCs were skin irritation, shortness of breath, runny nose, and coughing. In addition, a BTC also reported the staff discontent due to the stiffness of the Epimax container pump, causing a severe pain in staff fingers and the splash of disinfectant on donor and staff clothes, which left yellow stains after washing.

Table 1
Results of microbial culture before and after using CHG/IPA and the microbial flora diversity prior to disinfection of donors' arms.

Commercial name for disinfection spray	Sample size	Positive rate for pre-disinfection swabs (%)	Microbial flora for pre-disinfection swabs					Positive rate for post-disinfection swabs (%)
			Gram Positive Bacilli	Gram Positive Cocci		Gram Negative Bacilli	Undetermined	
			Corynebacterium	*S. epidermidis*	*Streptococci*	*Klebsiela*		
BodyPrep[a]	$N = 100$	100 (100)	2	79	16	0	3	0 (0)
Epimax[a]	$N = 100$	100 (100)	2	94	1	0	3	0 (0)
Germo[b]	$N = 100$	100 (100)	2	95	0	1	2	0 (0)
Total	300	300 (100)	6	268	17	1	8	0 (0)

[a] Product made by Iranian companies.
[b] Product made by Italian company.

Table 2
Results of skin aerobic cultures in the studied BTCs.

Yearly monitoring	Total donation No. per year[a]	1st six-month period		2nd six-month period		12-month period	
		Total No. of swab cultures	No. of positive culture (% per total cultures)	Total No. of swab cultures	No. of positive culture (% per total cultures)	Total No. of swab cultures (% per donation no)	No. of positive culture (% per total cultures)
2012 for PI[c]	2,042,315	1930	288 (14.92%)	2292	254 (11.08%)	4222 (0.21%)	542 (12.84%)
2013 for PI	2,001,791	2851	163 (5.72%)	3075	152 (4.94%)	5926 (0.30%)	315 (5.32%)
2014 for PI	2,071,031	2996	134 (4.47%)	3413	104 (3.05%)	6409 (0.31%)	238 (3.71%)
2015 for PI	2,083,914	3796	116 (3.06%)	5049	202 (4.00%)	8845 (0.42%)	318 (3.60%)
2016 for PI	2,127,064	5376	151 (2.81%)	5365	128 (2.39%)	10,741 (0.50%)	279 (2.60%)
2017 for PI	2,093,197	6953	137 (1.97%)	–	–	15,531 (0.74%)	297 (1.91%)
2017 for CHG/IPA[b]		–	–	8578	160 (1.87%)		

PI: povidone iodine; CHG/IPA: 2% chlorhexidine gluconate in 70% isopropyl alcohol.
[a] Total blood donation is for all BTCs per year.
[b] The monitoring of CHG/IPA just was done in second six-month of 2017.
[c] Due to the high rate of positive cultures in 2012, this data was not included in the analysis.

Table 3
The positive microbial culture ratios in summer and winter from 2013 to 2016 in the studied BTCs.

Yearly monitoring	Summer season		Winter season	
	Total no. of swab cultures	No. of positive culture (% per total cultures)	Total no. of swab cultures	No. of positive culture (% per total cultures)
2013 for PI	1501	75 (4.50%)	1305	86 (6.60%)
2014 for PI	1669	44 (2.67%)	1329	77 (5.80%)
2015 for PI	2857	149 (5.22%)	1600	42 (2.63%)
2016 for PI	2730	77 (2.82%)	2611	108 (4.14%)
2017				
PI	–	–	2829	73 (2.58%)
CHG/IPA	4320	82 (1.90%)	–	–
Total (with 2017 results)	13077	383 (2.93%)	9674	386 (3.99%)
Total (without 2017 results)	8757	345 (3.94%)	6845	313 (4.57%)

PI: povidone iodine; CHG/IPA: 2% chlorhexidine gluconate in 70% isopropyl alcohol.

4. Discussion

In the current study, a variety of Gram-positive bacteria such as *S. epidermidis* and some less common species such as *Streptococcus* spp., *Corynebacterium* spp., and *Bacillus* spp. were identified from the pre-donation skin cultures of participant; that were the normal flora of the skin and had been previously reported by earlier studies [17–19]. *S. epidermidis* had the highest frequency (nearly 90%), and was observed to be completely (100%) remove by all the disinfectant agents tested in the study. The six-month follow-up results showed that the use of CHG/IPA had a low positive rate (1.87%) and almost confirmed the result of the evaluation study.

Similar to earlier studies [1,9,19], the current study suggests that CHG/IPA method may be optimal in a blood services setting. For example, as noted by Ramirez–Arcos and Goldman [1], and, McDonald et al. [14], all methods tested in their studies had a 99% and 99.91% reduction of skin flora after disinfection with CHG/IPA, respectively. A remarkable reduction of skin flora after CHG/IPA disinfection was also reported by Wong et al. [9].

Many variables noted in the literature could influence the antiseptic efficacy and result in the difference [1,20]. It is believed that the employed mode of application (swab, gauze, scrub, applicator, or spray), one or two-step disinfection method, and donor features in the current study could be the main reasons for the remarkable reduction in bacteria load rather than other reasons. Since the bacteria residing deep in the skin layers are not accessible to disinfectants, [20] an initial hand scrubbing technique with sterile gauze helps to improve the effectiveness of disinfectant, particularly for the donors with more contamination in the venipuncture sites due to the difference in donor's features, occupation, and the amount of hair on the arm. This fact was quite tangible both in the evaluation study and effectiveness monitoring at BTCs.

The six-month follow-up of CHG/IPA with a sample size of 8578 donors confirmed that CHG/IPA was at least as effective as PI. Although the analysis of CHG/IPA results compared with those of PI in the last year could suggest the higher efficacy of the new disinfection technique, the decreasing trend in the positive rate of PI from 2012 to 2016 should not be ignored, and it appears that the difference was not significant. Similarly, several other studies suggest that CHG/IPA could be as optimal as PI [1–3,8–10,16].

Based on these results, the IBTO introduced the 2% CHX/IPA as the recommended solution for skin disinfection in blood collection procedure at BTCs. Beside the results of CHG/IPA, the rate of positive skin cultures for PI showed a reducing trend over the last years. There was an improvement in the positive rate of bacterial culturing by a well-managed corrective action and preventive action (CAPA) process like training programs and even more audits at BTCs. In addition, the change in the standard operative procedure, further supervision of the provision of PI solutions, the increase in QC sample size of skin cultures according to the frequency of blood donation in each center, and more supervision on the inclusion of previously neglected mobile and collection centers in QC sampling led to a tangible increasing trend in the number of QC samples. This factor could be another reason to observe the decreasing trend in the rate of positive skin cultures at BTCs.

Despite a report by Cid et al. [21] that revealed a more bacterial resistance in summer than winter, the current study found a more positive rate after disinfection in winter than summer; a surprising finding. However, no correlation was observed between the overall results of the first six-months to the second six-months, and these results still need to be confirmed and there is a need for further investigation.

Finally, no significant complaint or occurrence of allergy to CHG/IPA was observed in the scale of evaluation study, and the

results were similar to those reported by earlier studies [8,9,14]. CHG/IPA complaint reports in the routine operation at BTCs such as skin irritation, shortness of breath, coughing, and a runny nose were generally slight and self-limiting, without mentioning details of the subjects such as the frequency of incidents or complaints, the age and gender, and the donation history as first time or regular; hence, it could not be easily interpreted. Similar reports of skin irritation and allergic reactions were also reported by previous studies [1–3,10]. In this study, similar to the earlier reports [15], the CHG/IPA method was proved for being easy-to-use and evaporated well, which could be regarded as an appropriate antiseptic method for skin disinfection in blood donation sessions.

5. Conclusion

By comparing the results of CHG/IPA with those of PI during the six-month period, it was ensured that CHG/IPA had a disinfection efficacy equivalent to that of the previous "best-practice" technique. The results provided by the current study in BTCs demonstrated that the new disinfectant can resolve the concerns related to preparing a 0.7% PI solution, and that it is easy-to-use for staff and acceptable to the donors.

Disclosure of interest

The authors declare that they have no competing interest.

Authors contributions

Mohammad Hessam Rafiee: Experimental research and writing the manuscript.

Sedigheh Amini Kafiabad: Study design and supervision.

Mahtab Maghsudlu: Statistical analysis.

Mohammad Moradi: Experimental research and writing the manuscript.

Lida Jalili: Experimental research.

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