Fluconazole Effectiveness in Preventing Invasive Fungal Infection in Very Low Birth Weight Infants: Systematic Review and Meta-analysis

(Efektivitas Flukonazol dalam Mencegah Infeksi Jamur Invasif pada Bayi dengan Berat Lahir Sangat Rendah: Tinjauan Sistematis dan Meta-analisis)

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Abstract: Fungal infections in neonates, especially in Very Low Birth Weight (VLBW) infants, are mostly caused by Candida species and may lead to morbidity and mortality. A systematic review and a meta-analysis were conducted to determine the extent to which fluconazole, an antifungal prophylactic, was effective and safe to use in VLBW or premature infants in preventing Invasive Fungal Infection (IFI), by including Randomized Controlled Trials (RCTs) carried out worldwide. The investigation started with searching process through publication databases: MEDLINE, Cochrane, ScienceDirect, and Garuda, for Randomized Controlled Trials (RCTs) that compared the prophylactic effects of fluconazole and placebo on IFI in VLBW infants. The selected eight RCT studies indicated that, compared to placebo, fluconazole accounted for 68% risk reduction of overall fungal colonization (RR=0.32; 95% confidence interval [CI]=0.24-0.42, p=0.00001, I-square=0%) and 60% risk reduction of IFI (RR=0.40; 95%[CI]=0.22-0.72, I-square=56%, p=0.002). However, fluconazole did not significantly reduce mortality in VLBW infants (RR=0.79; 95%[CI]=0.60-1.03; p=0.08, I-square=0%). Also, regarding its safety, fluconazole prophylaxis did not result in significant elevations of SGOT/SGPT levels (RR=1.22; 95%[CI]=0.50-3.00, p=0.66, I-square=0%) nor cause intestinal perforation (RR=0.96; 95%[CI]=0.25-3.68, p=0.96, I-square=59%). Fluconazole is an effective prophylaxis agent against invasive fungal infection when given to preterm infants with birth weight <1500, but not proven in reducing the mortality incidence in VLBW infants.

Keywords: antifungal, fluconazole, infant, invasive fungal infection, prevention and control, very low birth weight.

Abstrak: Infeksi jamur pada neonatus, terutama pada bayi dengan Berat Badan Lahir Sangat Rendah (BBLSR), sebagian besar disebabkan oleh jamur jenis Candida, dan dapat menyebabkan morbiditas dan mortalitas. Kami melakukan tinjauan sistematis dan meta-analisis untuk melihat besarnya efektivitas dan keamanan, khususnya penggunaan flukonazol sebagai profilaksis infeksi jamur invasif pada BBLSR atau bayi prematur menggunakan Randomized Controlled Trials (RCT) secara global. Peneliti mencari melalui MEDLINE, Cochrane, ScienceDirect, dan Garuda untuk mengidentifikasi RCT yang membandingkan efek profilaksis infeksi jamur invasif dari flukonazol dan placebo pada bayi BBLSR. Delapan artikel RCT yang dimasukkan dalam meta-analisis ini menghasilkan 68% pengurangan risiko kolonisasi jamur (RR=0.32; interval kepercayaan 95% [CI]=0.24-0.42, p=0.00001, I-square=0%) dan 60% pengurangan risiko infeksi jamur invasif (RR=0.40; CI95%=0.22-0.72, p=0.002, I-square=56%) dengan penggunaan flukonazol. Namun demikian, flukonazol tidak signifikan menurunkan angka kematian pada bayi BBLSR (RR=0.79; CI95%=0.60-1.03; p=0.08, I-square=0%). Berkaitan dengan masalah keamanan, tidak terjadi elevasi SGOT / SGPT (RR=1.22; CI95%=0.50-3.00, p=0.66, I-square=0%) dan perforasi usus (RR=0.96; CI95%=0.25-3.68, p=0.96, I-square=59%) yang signifikan pada kelompok flukonazol. Flukonazol merupakan agen profilaksis yang efektif terhadap infeksi jamur invasif bila diberikan pada bayi prematur dengan berat lahir <1500 g, namun belum terbukti bermanfaat dalam mengurangi kematian pada bayi BBLSR.

Kata kunci: antijamur, bayi, berat badan lahir sangat rendah, flukonazol, infeksi jamur invasif, pencegahan dan pengendalian.

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INTRODUCTION

FUNGAL infections in neonates are caused chiefly by Candida species\(^1\). Candida, a genus of yeasts, can live in the skin, throat, vagina, and esophagus and often survives in the human body without causing any diseases or problems. However, a fungal infection in premature or Very Low Birth Weight (VLBW) infants can be fatal. Premature infants admitted to the Neonatal Intensive Care Unit (NICU) are at significant risks of contracting a fungal infection, which, according to studies, has an incidence rate of up to 50%. Further, it can develop into an invasive fungal infection (IFI), which is a systemic infection that potentially leads to morbidity and mortality.

Therefore, many NICU healthcare professionals are looking for a solution to prevent fungal colonization from occurring in infants by, among others, administering fluconazole. Fluconazole is widely used in treating infections of various types of fungi, including the Candida type. Fluconazole is not only used for medicinal purposes. It is also used in preventing the colonization of fungi\(^2\,3\). Several studies have compared levels of effectiveness of fluconazole in the prophylaxis of invasive fungal infections due to various Candida species, namely Candida albicans and other Candida fungi. In those studies, fluconazole has been found to effectively reduce colonization in different parts of the human body, such as the skin, digestive tract, and respiratory tract. Up to now, systematic reviews and meta-analysis have been conducted to see the effectiveness of antifungal generally, while other studies focusing on fluconazole use only in a specific region (United States of America)\(^4\,5\).

McQuire, et al.\(^6\) studied meta-analysis of fluconazole usage as preventive in VLBW infants using before-after study design and found that publication bias may occur using this study design, compared to Randomized Controlled Trial (RCT) design\(^7\). Therefore, the current research conducted both systematic review and meta-analysis to determine the extent to which fluconazole was effective and safe to use in the prophylaxis of IFI in VLBW or premature infants by pooling individual RCTs worldwide.

METHODS

We analyzed RCT comparing the prophylactic effect of fluconazole and placebo or no drug in very low birth weight infants weighing <1500 g at birth. This study used two different groups: the group using fluconazole and the group using a placebo.

Data Sources and Searches. We examined all data from references using RCT for fluconazole prophylactic effect in VLBW or preterm infants. We searched through four databases, in MEDLINE using keywords of the following MeSH terms: ((“Premature Birth”[Mesh]) OR “Infant, Very Low Birth Weight”[Mesh]) AND “flucconazole”[Mesh]) AND “prevention and control”[Subheading]; database Cochrane Library using keywords of the following text words: Fluconazole AND very low birth weight infant AND prevention; database ScienceDirect using keywords of the following text words: Fluconazole AND very low birth weight infants AND prevention and control; and database Garuda using keywords of the following text words: fluconazol DAN bayi DAN pencegahan. We also searched for articles manually, through related research articles, related reviews, and also looked for relevant proofreading. We did not apply any language and year of publication restrictions.

Study Selection. The individual RCTs obtained from the database searches were then selected using several inclusion criteria. First, the participants consisted of VLBW infants weighing less than 1500 g or preterm infants born at less than 34 weeks. Second, the intervention was with fluconazole prophylaxis through the intravenous route, enteral route, or both. In addition, the primary outcomes evaluated in the study were fungal colonization, confirmed invasive fungal infections, and mortality, while the secondary outcomes were elevated SGOT/SGPT levels and intestinal perforation. Meanwhile, irrelevant studies and major uncertainty caused by methodological weaknesses were excluded from meta-analysis.

Data Extraction and Quality Assessment. Three investigators assessed the suitability of the topic on the abstracts and then checked the quality of the trials using the The Critical Appraisal Skills Program (CASP) checklist for RCT studies. A good quality trial means all the criteria are met. When the criteria are not fully met but are classified as almost fully met, it can be said that the quality of the study is fair. Thus, studies that are classified as poor are those that hardly or do not meet the criteria.

Data Synthesis and Analysis. A meta-analysis was conducted to identify the effects and effectiveness of fluconazole from various RCT studies, as evidenced by the overall incidence of fungal colonization (at least at one site), invasive fungal infections, death due to any cause, and side effects like increased SGOT/SGPT levels and intestinal perforation. The effectiveness was evaluated using the RevMan v.5.4 program. Also, the relative risk for all cases was analyzed based on
the number of events and the absence of events. The Mantel-Haenszel random effect method was used to generalize the results globally. In addition, a sensitivity analysis was performed to evaluate the effectiveness of fluconazole prophylaxis in all of the included trials and compared to excluding those with poor or unidentified quality. Subgroup analysis was conducted on dose variety and infants with Extremely Low Birth Weight (ELBW), i.e., less than 1,000 g.

RESULTS AND DISCUSSION

Global searches through the databases and other sources came back with 35 studies and one study, respectively. However, among these 36 studies, two were duplicates and were hence removed. Then, of the 32 studies, 23 were irrelevant for the meta-analysis (as shown by the abstracts) and were thereby excluded. Eventually, as seen in Figure 1, eight RCT studies, including one conference abstract, were analyzed qualitatively and quantitatively in the current research. In those studies, the trial population size ranged from 26 to 361 infants with a birth weight of <750 to <1,500 g, and the fluconazole was administered at different dosages: from 3 to 6 mg/kg twice weekly or every 24 hours for 28 to 42 days\(^{(8-15)}\). The characteristics of the eight RCTs are summarized in Table 1.

**Qualitative Assessment.** We identified the quality of each paper included using the CASP checklist. From the eight RCT trials, 7 had acceptable quality, and 1 trial from the conference abstract was unidentified. The significant limitations of trials included, which might lead to bias, can be seen in Table 2, and the summary of quality assessment can be seen in Figure 2.

The meta-analysis of eight individual RCTs revealed that, compared with the placebo group, fluconazole could significantly reduce overall fungal colonization (RR=0.32; 95% confidence interval [CI]=0.24 to 0.42, \(p<0.00001\), I-square=0%, Figure 3) and invasive fungal infections (RR=0.40; 95% confidence interval [CI]=0.22 to 0.72, \(p=0.002\), I-square=56%, Figure 4) but had no significant effect on mortality (RR=0.79; 95% confidence interval [CI]=0.60 to 1.03, \(p=0.08\), I-square=0%, Figure 5). The subgroup analysis of ELBW infants also showed similar results on invasive fungal infections (RR=0.30 95%; confidence interval [CI] 0.13 to 0.70, \(p=0.005\), I-square = 0%).

![Figure 1. Provisional PRI.](Figure 1. Provisional PRI.)
Table 1. General characteristics of the included studies.

<table>
<thead>
<tr>
<th>Study (Reference)</th>
<th>Study Design</th>
<th>Dose</th>
<th>Treatment Duration</th>
<th>Participants, n</th>
<th>IFI Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirpal et al.(^8)</td>
<td>RCT</td>
<td>6 mg/kg</td>
<td>28 days or discharge whichever was earlier</td>
<td>80 infants with birth weight &lt; 1500 g</td>
<td>Candida isolation from blood and/or cerebrospinal fluid.</td>
</tr>
<tr>
<td>Benjamin et al.(^9)</td>
<td>RCT</td>
<td>6 mg/kg of body weight, twice weekly</td>
<td>42 days</td>
<td>361 with birth weight of less than 750 g.</td>
<td>Definite candidiasis was defined as a positive Candida culture from a normally sterile body fluid such as blood, cerebrospinal fluid, peritoneal fluid, or urine obtained via suprapubic aspiration or in/out catheterization. Probable invasive candidiasis was defined as receipt of more than 5 days of consecutive antifungal therapy and both thrombocytopenia (&lt;150,000/103 μL) and a positive Candida culture from a non-sterile site (e.g., bag urine).</td>
</tr>
<tr>
<td>Aydemir et al.(^10)</td>
<td>RCT</td>
<td>3 mg/kg body weight, every third day</td>
<td>30 days (45 days for neonates weighing)</td>
<td>184 infants with birth weight less than 1500 g</td>
<td>A positive culture from the blood (peripheral venipuncture), urine (≥10,000 or more colony-forming U/ml from sterile bladder catheterization or suprapubic aspiration), or cerebrospinal fluid.</td>
</tr>
<tr>
<td>Parikh et al.(^11)</td>
<td>RCT</td>
<td>6 mg/kg/day as a single dose every 72 hours till day 7 and subsequently every 24 hours</td>
<td>28 days or less if, discharged or died earlier</td>
<td>120 infants with birth weight 1500 g</td>
<td>Fungal growth in blood</td>
</tr>
<tr>
<td>Manzoni et al.(^12)</td>
<td>RCT</td>
<td>either 6 mg or 3 mg per kilogram of body weight</td>
<td>30 days (45 days for neonates weighing)</td>
<td>336 infants with birth weight less than 1500 g</td>
<td>A positive culture from the blood (a peripheral site), urine (collected by sterile suprapubic puncture or bladder catheterization, with the growth of ≥10,000 organisms per milliliter), or cerebrospinal fluid</td>
</tr>
<tr>
<td>Kaufman et al.(^13)</td>
<td>RCT</td>
<td>3 mg per kilogram of body weight every third day</td>
<td>6 weeks</td>
<td>100 infants with birth weight less than 1000 g</td>
<td>A positive culture of blood, urine, or cerebrospinal fluid</td>
</tr>
</tbody>
</table>
Table 2. Majority limitations of papers included.

<table>
<thead>
<tr>
<th>Checklist</th>
<th>Limitations</th>
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<tbody>
<tr>
<td>Clearly focused issue</td>
<td>Based on the exclusion criteria of 1 paper, there is a chance of patients with IFI before enrollment. This paper did not mention the baseline criteria for any colonization or IFI events.</td>
</tr>
</tbody>
</table>
| Allocation concealment                       | ● Two papers did not give enough information, which may lead to the risk of bias during the allocation process  
  ● One unclear piece of information from the conference abstract.                                                                                   |
| Blinding                                     | ● No detailed description of the blinding mechanism in the four papers, 1 of them provided different appearances of drugs, and it’s impossible to do the blinding.  
  ● One unclear piece of information from the conference abstract.                                                                                   |
| The similarity of two groups at the beginning | ● In 1 paper, the use of corticosteroids is significantly higher in the fluconazole group (p<0.05). In 1 other paper, there were differences in steroid use and cesarean section. In the end, the multivariate analysis had been done to adjust this confounding.  
  ● One unclear piece of information from the conference abstract.                                                                                   |
| Treatment equality                           | One unclear piece of information from the conference abstract.                                                                                                                                               |
| Intention-to-treat                           | ● One paper had incomplete data due to a randomization error that occurred  
  ● One other paper had 14 incomplete data, including four incorrect drug administrations.  
  ● One unclear piece of information from the conference abstract.                                                                                    |
| Selective-reporting possibility              | None                                                                                                                                                                                                       |
| The precision of treatment effects           | Three papers did not mention confidence interval level.                                                                                                                                                      |

![Quality Assessment of 7 RCT Papers](image)

Figure 2. Summary of quality assessment.

![Forest plot](image)

Figure 3. Forest plot of the efficacy of fluconazole compared to placebo based on overall fungal colonization (at least one site). M-H, Mantel-Haenszel; CI, confidence interval.
Safety of Fluconazole. Fluconazole is considered safe to use for VLBW or premature infants. It was concluded from the absence of any significant elevation of SGOT/SGPT levels (RR=1.22; 95% confidence interval [CI]=0.50 to 3.00, p=0.66, I-square = 0%, Figure 6) and intestinal perforation (RR = 0.96; 95% confidence interval [CI]=0.25 to 3.68, p=0.96, I-square = 59%, Figure 7) in the two groups.
Subgroup and Sensitivity Analysis. A subgroup analysis was also performed on different doses to overcome heterogeneity. It revealed that the doses 3 mg/kg and 6 mg/kg showed similar primary and secondary outcomes. At 6 mg/kg, fluconazole administration was associated with a relative risk (RR) of 0.29 for overall fungal colonization (95% confidence interval [CI] 0.20 to 0.42, p<0.00001, I-square=0%), RR=0.21 for invasive fungal infections (95% confidence interval [CI]=0.10 to 0.45, p<0.0001, I-square=0%), and RR=0.66 for deaths (95% confidence interval [CI]=0.39 to 1.13, p=0.13, I-square=0%). Similarly, when given at 3 mg/kg, it was associated with RR=0.34 for overall fungal colonization (95% confidence interval [CI]=0.24 to 0.50, p=0.00001, I-square=0%), RR=0.52 for invasive fungal infections (95% confidence interval [CI]=0.36 to 0.76, p=0.0006, I-square=47%), and RR=0.81 for deaths (95% confidence interval [CI]=0.60 to 1.10, p=0.18, I-square=0%).

Excluding one conference abstract, the sensitive analysis revealed no different results from the eight RCT studies for all the primary and secondary outcomes, i.e., overall fungal colonization (RR=0.31; 95% confidence interval [CI]=0.24 to 0.41, I-square=0%, p<0.0001), invasive fungal infections (RR=0.42; 95% confidence interval [CI]=0.30 to 0.59, p<0.0001, I-square=61%), mortality (RR=0.78; 95% confidence interval [CI]=0.60 to 1.03, p=0.08, I-square=0%), elevation of SGOT/SGPT levels (RR=1.33; 95% confidence interval [CI]=0.56 to 3.19, p=0.52, I-square=0%), and intestinal perforation (RR=1.21; 95% confidence interval [CI]=0.61 to 2.38, p=0.59, I-square=59%).

This review included eight RCTs that assessed the benefits and harms of using fluconazole as a prophylactic agent against fungal colonization, invasive fungal infections, and all-cause mortality in a total participant of 1,310 VLBW infants. Consistent evidence with previous systematic reviews and meta-analyses was found. McQuire et al. reviewed eleven before-and-after studies and pointed out a significant reduction of invasive fungal infections in the fluconazole group. However, a small study size with large effects from these observational studies may contribute to publication bias. Cleminson et al. also identified a significant reduction of invasive fungal infections (RR=0.43, 95% confidence interval [CI]=0.31–0.59) in the antifungal group and no significant all-cause mortality (RR=0.79, 95% CI=0.61–1.02) in the antifungal and placebo groups; the antifungals were fluconazole and oral non-absorbed agents, such as nystatin and miconazole. Reviewing four RCTs conducted in the United States, Ericson et al. found that the fluconazole group exhibited significantly reduced fungal colonization and invasive candidiasis, but the contrary for mortality. The odds ratio of these three outcomes were 0.28 (95% CI=0.18–0.41), 0.20 (95% CI=0.08–0.51), and 0.68 (95% CI=0.40–1.13).

In terms of safety, Ericson et al. suggested that fluconazole was safe to use as a prophylactic agent in VLBW infants, as indicated by the absence of differences in the elevation of SGOT/SGPT levels and intestinal perforation between the fluconazole and placebo groups (P>0.05 for all events). Furthermore, no significant difference was found in the other safety events measured, i.e., necrotizing enterocolitis, chronic lung disease, retinopathy, abnormal alkaline phosphatase, and abnormal direct bilirubin (P>0.05 for all events). Nevertheless, it has been reported that, with prolonged treatment, the use of this antifungal could develop into cases with fluconazole-resistant Candida species through various mechanisms.

However, other studies such as Kaufman et al. and Manzoni et al. found that the Minimum Inhibitory Concentration (MIC) of fluconazole did not differ significantly throughout the 30 months of their study periods, suggesting no variability in the sensitivity patterns and resistances of the fungi observed to the.
medication\(^\text{(12,13)}\). Similarly, Kicklighter et al. also reported no significant differences in the MIC of the isolates between the fluconazole and the control groups during the treatment period\(^\text{(14)}\).

Of the eight RCTs, only two examined the effects of fluconazole on neurodevelopmental impairment. Benjamin et al. demonstrated no differences between the fluconazole and placebo groups regarding neurodevelopmental impairment, as evaluated in a follow-up at 18-22 months corrected age. Their study defined developmental impairment as a Bayley-III cognitive scale of less than 70\(^\text{(9)}\).

Correspondingly, Kaufman et al. found that the developmental impairment did not differ between the groups receiving fluconazole prophylaxis and placebo after evaluating the enrolled premature infants at 8-10 years of age using the Vineland Adaptive Behavior Scales-II (VABS-II). Further, using the Child Health Questionnaire for Parents-Completed Form 28 (CHQ-PF28), it was concluded that their quality of life also did not differ significantly\(^\text{(17)}\). As evident in these trials, fluconazole is considered safe for antifungal prophylaxis because it is not linked to any long-term effects on neurodevelopment. The trials included in this study prescribed two different regimens of fluconazole prophylaxis: 3 mg/kg and 6 mg/kg. Based on the subgroup analysis results, there were no differences between their clinical outcomes. In 2005, Kaufman et al. found that, instead of the regular dosing schedule, a twice-weekly regimen may be beneficial in lowering not only Candida colonization and invasive fungal infection in infants weighing less than 1,000 g at birth but also cost\(^\text{(18)}\).

In 2012, Manzoni et al. revealed baseline characteristics of the enrolled VLBW infants as a potential contributing factor to the results of prophylactic fluconazole regimens. Infants with baseline and actual colonization might show a different progression of fungal infection and a different response to the fluconazole regimen\(^\text{(19)}\). However, not all of the trials included in this study factored in and discussed the baseline criteria for colonization or IFI events.

Aside from the revelation of baseline characteristics in only some of the included trials, this review also has another limitation related to unexplained heterogeneity. Subgroup analysis was performed for the different doses used in the selected trials, but the root of the heterogeneity in the invasive fungal infection data was unable to be identified. Moreover, most of the trials included a follow-up less than a decade after the completion, making it difficult to distinguish the effects of fluconazole on neurodevelopmental impairment and quality of life in the long term.

**CONCLUSIONS**

Fluconazole is an effective prophylaxis agent against invasive fungal infection and fungal colonization when given to preterm infants with birth weight <1500 g. Fluconazole does not reduce the incidence of mortality in VLBW infants.

**REFERENCE**