INTRODUCTION

Erectile dysfunction (ED) is a persistent or recurrent inability to obtain or maintain a penile erection sufficient for satisfactory sexual intercourse. The prevalence of ED increases with age, and in general, ED is less than 10% in men < 40 years, less than 15% in 40–49 years, 20–30% in 50–69 years, 20–40% at age 60–69 years, and 50–100% at age 70 years. The risk factors for ED can be classified into four categories, (a) cardiovascular and metabolic disorders (such as diabetes mellitus, hypertension, hyperlipidemia, and obesity), (b) andrological or urological diseases, (c) psychosomatic and psychiatric disorders and (d) lifestyle disorders (such as smoking and sedentary lifestyle). Penile erection is a complex phenomenon that requires vascular, neurologic, and tissue interactions. Consists of dilation of penile arteries and relaxation of the smooth muscle of the trabecular tissue accompanied by a mechanism of corporeal vein occlusion. Phosphodiesterase type 5 inhibitors (PDE5-I) have long been used as the mainstay of therapy for ED. PDE5-Is work by slowing the degradation of cyclic guanosine monophosphate (cGMP), which is a main regulator of intracellular calcium by type 5 phosphodiesterase. cGMP has an important role in smooth muscle relaxation and blood accumulation in the corpora cavernosa, which is required for penile erection.

The efficacy of PDE5-Is demonstrates the importance of the NO-cGMP signaling pathway through inhibition of NO-generated cGMP degradation. PDE5-Is selectively inhibit PDE5, a PDE5 inhibitor, thereby maintaining NO-triggered relaxation of cavernous trabecular tissue smooth muscle in cGMP. Tadalafil is a selective PDE5-I that was approved for clinical use in 2003 after the approval of sildenafil and vardenafil. Tadalafil has a rapid onset of action with a long duration of action (36 hours). The efficacy of tadalafil is not affected by dietary intake.

L-arginine is a supplement that is a major precursor of nitric oxide (NO), which is the most important vasoactive...
neurotransmitter required for penile smooth muscle relaxation. NO has an important role in improving endothelial function. L-arginine improves erectile function by increasing the endogenous amino acids required for ideal NO synthesis and production. In addition, NO affects the signaling of guanylyl cyclase-protein kinase G soluble in nitric oxidase, which includes stimulation of potassium-dependent calcium channels or preventing upregulation of the RhoA/Rho-kinase pathway. Dietary supplementation with L-arginine may play an important role in treating ED.

Several recent studies have shown that L-arginine, the main NO precursor that plays a role in the NO-cGMP signaling pathway in smooth muscle, causes an increase in cGMP, which results in penile smooth muscle relaxation. With smooth muscle relaxation, L-arginine can be used as a therapy option in ED. The combination of tadalafil and L-arginine can improve erectile function as assessed by the IIEF-5 score. Several studies have also reported that combination therapy with tadalafil and L-arginine can affect testosterone levels. This significant increase in testosterone levels still within physiological limits is said to occur through several mechanisms. PDE5-I can stimulate the expression of steroidogenic protein kinase G. At the same time, L-arginine, through increased NO concentration and cGMP release, causes vasodilation and improved blood flow to the testes, so that both can synergistically increase testosterone levels. Increased self-confidence, frequency of sexual intercourse, and quality of life accompanying increased erectile ability can indirectly stimulate testosterone secretion.

The efficacy of combination of tadalafil and L-Arginine has not been established. Therefore, we conducted a meta-analysis to determine the efficacy and safety of the combination therapy of tadalafil and L-arginine compared to tadalafil monotherapy for the treatment of ED.

**METHODS**

**Study Design**

This study uses a quantitative method that refers to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol. The independent variable in this study was the combination of tamsulosin, L-arginine (intervention), and tadalafil (control), while the dependent variable was the IIEF-5 score and testosterone levels.

**Systematic Search Strategy**

Study searches will be carried out systematically on several online databases, such as Embase, MEDLINE, and Scopus. The keywords used were “tadalafil”, “arginine”, “L-arginine”, and “erectile dysfunction” or “ED” (Table 1). Study search and selection were constructed according to PRISMA guidelines. This study’s protocol was registered on PROSPERO (CRD42022343590).

**Eligibility Criteria**

The inclusion criteria of this study consists of randomized controlled trials (RCTs), populations include adult patients with ED who were treated with tadalafil and L-arginine combination therapy and tadalafil monotherapy and can be accessed through Embase, MEDLINE, and Scopus. In addition, the exclusion criteria for this...
Table 2. Baseline characteristics of research articles.

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Group</th>
<th>Type of intervention</th>
<th>Δ Total testosterone</th>
<th>Δ IIEF-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>La Vignera et al., 2012</td>
<td>Tadalafil</td>
<td>EAC contains L-arginine (2500 mg), propionyl-L-carnitine (1250 mg), and nicotinic acid for 24 weeks plus tadalafil</td>
<td>NR</td>
<td>10 ± 2.88</td>
</tr>
<tr>
<td></td>
<td>Tadalafil + L-arginine</td>
<td>L-arginine 2500 mg plus tadalafil 5 mg every day for 12 weeks.</td>
<td>NR</td>
<td>7.1 ± 5.7</td>
</tr>
<tr>
<td>Gallo et al., 2020</td>
<td>Tadalafil</td>
<td>L-arginine 2500 mg plus tadalafil 5 mg every day for 12 weeks.</td>
<td>NR</td>
<td>10 ± 2.85</td>
</tr>
<tr>
<td></td>
<td>Tadalafil + L-arginine</td>
<td>L-arginine 5 g and tadalafil 10 mg every day for 8 weeks.</td>
<td>8.97 ± 2.71</td>
<td>7.63 ± 1.08</td>
</tr>
<tr>
<td>El Taieb et al., 2019</td>
<td>Tadalafil</td>
<td>L-arginine 5 g and tadalafil 10 mg every day for 6 weeks.</td>
<td>NR</td>
<td>12.92 ± 2.64</td>
</tr>
<tr>
<td></td>
<td>Tadalafil + L-arginine</td>
<td>L-arginine 5 g and tadalafil oral tablet and 5 mg tadalafil oral tablet for six weeks</td>
<td>11.23 ± 2.74</td>
<td>13.69 ± 2.1</td>
</tr>
<tr>
<td>El Hamid et al., 2019</td>
<td>Tadalafil</td>
<td>L-arginine 5 g and tadalafil oral tablet and 5 mg tadalafil oral tablet for six weeks</td>
<td>11.27 ± 1.7</td>
<td>12.27 ± 1.7</td>
</tr>
</tbody>
</table>

study were non-English language journals, experimental animal studies, unpublished articles, and abstract-only articles.

**Data Extraction**

Data extraction was carried out by two authors independently by filling in the specified template. If there is a difference in the data extraction results, it will be discussed and decided by the third author. The extracted data consisted of the characteristics and methodology of the study, namely, the name of the first author, year of publication, number of patients, age of the patient, and study design. Then there are research interventions divided into the type of intervention given, a combination of tadalafil and L-arginine or tadalafil monotherapy. The extracted outcomes were the IIEF-5 score and testosterone levels.

**Quality Assessment**

The risk of research bias in this study was assessed using the Cochrane Risk of Bias Tools for randomized trials. Five components were evaluated, including the randomization process, bias resulting from deviation from the intervention, bias resulting from insufficient outcome data, bias resulting from outcome measurement techniques, and bias resulting from outcome selection and reporting.

**Statistical Analysis**

Continuous outcome data were analyzed to produce a mean difference with a confidence interval of 95%, and a p-value below 0.05 was determined as statistically significant. Heterogeneity between studies was calculated using I². If I² > 50%, it is considered that heterogeneity between studies is statistically high, and random effects model analysis will be used in the meta-analysis. If I² < 50%, the fixed effect model will be applied to the meta-analysis. Software RevMan 5.4 for Windows is used as a data analysis tool in this study. The analysis results are presented in forest plots and descriptive narratives.

**RESULTS**

**Systematic search results**

Based on the PRISMA flow guide in Figure 1, the search results on several databases, such as Pubmed, Scopus, and EMBASE,
yielded 81 articles according to the study search keywords. There were about 11 duplicate studies excluded. Furthermore, screening was carried out through titles and abstracts, which resulted in 70 articles. A total of 37 articles were excluded based on selecting irrelevant titles and abstracts. Of the 33 articles assessed for full-text, 29 were excluded because of the wrong intervention, comparison, and population. Four articles were produced for further qualitative and quantitative analysis.

Baseline characteristics of the included studies
The characteristics of each study included in the study are listed in Table 2. This meta-analysis included studies evaluating comparisons between tadalafil monotherapy and tadalafil and L-arginine combination therapy in patients with ED. The study in this study was published between 2011 and 2020. The data extracted were the name and year of the study author, research design, number of samples, intervention, and the average age of the sample, which were entered into the tabulation of the baseline characteristics in this study. Outcomes were assessed through testosterone levels and IIEF-5 scores and entered into the tabulation of study outcomes. The total number of patients analyzed in this meta-analysis reached 364, consisting of 182 patients on tadalafil therapy and 182 on tadalafil plus L-arginine therapy. All studies included in this study were RCTs.

Risk of bias assessment
Research bias assessment was carried out on four studies which were analyzed qualitatively and quantitatively. The Cochrane Risk of Bias Tools assessed this study's randomized controlled trial studies (RCTs). In general, the research conducted by La Vignera et al. and El-Hamd et al. had moderate research bias. In the research of La Vignera, there is a bias in the missing outcome data and attention to the bias in allocation concealment. While in El-Hamd's research, attention to the bias in allocation concealment, missing outcome data, and selective reporting. El-Taieb et al. and Gallo et al. have low research biases (Figure 2).

Outcome on the IIEF-5 score
The forest plot analysis in this study also evaluated the differences in IIEF-5 in the two study groups. From the results of the analysis of these four studies, there are significant differences in the IIEF-5 score in the two treatment groups (p < 0.00001; MD 1.64; 95% CI: 1.13-2.15), which is described in Figure 3. The fixed-effects model was used in this analysis because of

Figure 2. Risk of research bias with risk of bias in randomization trial (ROB).

Figure 3. Forest plot comparison of mean IIEF-5 scores in the two groups.

Figure 4. Forest plot comparison of mean testosterone levels in the two treatment groups.
the low heterogeneity between studies (I²: 59%).

**Outcome on the testosterone level**

This meta-analysis analyzed the difference in testosterone levels in administering tadalafil monotherapy with tadalafil plus L-arginine. From the results of the analysis of 2 studies (El-Hamd, 2020 and El-Taieb, 2019) that were included in this meta-analysis, there were significant differences in testosterone levels (p < 0.00001; MD 3.05; 95% CI: 2.05-4.04) which is described in Figure 4. The fixed-effects model was used to analyze this outcome because the heterogeneity between studies was moderate with an I² of 67%.

**DISCUSSION**

This study is the first systematic review and meta-analysis of the efficacy of tadalafil and L-arginine combination for ED that discusses testosterone levels and IIEF-5 subdomain scores (overall satisfaction, sexual satisfaction, sexual desire, orgasmic function, and erectile function). The results showed that additional arginine supplementation, compared with tadalafil monotherapy, provided a significant benefit in both testosterone levels and IIEF-5 scores. This study showed significant results from the administration of tadalafil in combination with L-arginine compared to tadalafil alone in increasing the IIEF-5 score in ED patients. Testosterone levels also showed a significant increase.

Daily oral administration of 5 g of L-arginine for six weeks can dramatically improve Sexual Health Inventory for Men (SHIM) scores and total testosterone levels in male patients with ED who are at least 60 years of age. This result is in line with Chen et al. who found that daily use of L-arginine in ED patients dramatically increased blood testosterone levels and improved erectile performance.

In addition, another study showed that daily oral treatment of 5 g of L-arginine for four weeks significantly increased testosterone levels, erectile function, sexual desire, sexual satisfaction, and sexual pleasure in diabetic men with ED. According to a recent study by El Taieb, injection of arginine 5 g daily for eight weeks significantly improved IIEF-5 scores and total testosterone levels in male patients with diabetic ED. This result is corroborated by Huang et al. who found that endothelial function in ED patients, erectile hardness, and IIEF-5 scores significantly improved after taking tadalafil 5 mg daily for 6-8 weeks. A study by Li et al. found that a tadalafil dose of 5 mg daily for 12 weeks significantly improved IIEF-5, relationship questionnaire, and self-esteem. According to a study by Ozcan et al., an increase in total testosterone levels resulted from the daily use of 5 mg of tadalafil, which greatly improved ED symptoms.

PDE5-I is currently the first line of treatment for ED. However, L-arginine supplementation may become an alternative for patients with mild to moderate ED since it is more psychologically acceptable as it is considered a supplement rather than a drug. Cormio et al. showed that all patients who reported an increase in erectile hardness score after citrulline administration continued with the same treatment rather than requesting PDE5-I. The results of our analysis show that patients with ED have many advantages over L-arginine combination therapy in combination with PDE5-I.

A recent study showed that most ED patients had low arginine or citrulline levels. In particular, this condition is more common in patients with an arteriogenic etiology. In addition, it was reported that higher homocysteine levels were frequently observed in patients with ED. In an animal model study, Jones et al. found that hyperhomocysteinemia promoted a marked inhibitory effect on NO formation in the isolated corpus cavernosum. This study found that homocysteine directly inhibits the activity of dimethylarginine dimethylaminohydrolase, an enzyme that degrades asymmetric dimethylarginine. Asymmetric dimethylarginine is an endogenous NO inhibitor and can accumulate in hyperhomocysteinemia. Based on these results, it can be postulated that high homocysteine levels may be an independent risk factor for ED by interfering with the NO pathway. A phenomenon in other diseases associated with high homocysteine levels, oral arginine supplementation has been shown to reduce its effects. The results showed that increasing levels of NO substrates could help overcome the inhibitory effect caused by homocysteine. Further studies should investigate the possible role of arginine in the presence of high homocysteine levels on the effectiveness of tadalafil and arginine.

The exact mechanism of increased total serum testosterone levels in combination with L-arginine remains unclear. However, it could be related to L-arginine supplementation, which improves erectile function and thereby increases confidence in sexual activity. This positive response increases testosterone secretion from the Leydig cells of the testes. L-arginine can increase NO synthesis and release and testicular blood flow, increasing testosterone synthesis and secretion and improving sexual performance.

Another study found that increasing NO concentration increased the release of cGMP, a vasodilator that increases testicular blood flow, testosterone synthesis, and secretion in Leydig cells. NO increases penile vasodilation and blood flow by diffusing smooth muscle membranes and stimulating soluble guanylate cyclase from corpus cavernosum smooth muscle to produce cGMP, which leads to an enzymatic cascade, which prevents calcium influx, reduces cytosolic calcium concentration, and thereby promotes cavernous smooth muscle relaxation.

Another study related to other PDE5-I used the combination of sildenafil and L-arginine compared with sildenafil alone in the treatment of ED. Primary takeaways from this study align with our study’s results, where the combination of L-arginine and sildenafil showed higher benefit compared to sildenafil alone for treating ED. Articles on the combination of sildenafil and L-arginine is still very limited. In addition, there are studies regarding the effect of propionyl-L-carnitine, L-arginine, and nicotinic acid on the efficacy of vardenafil in the treatment of ED in diabetic patients, with the result that this combination can improve the endothelial function among people with diabetes. The combination therapy with vardenafil was superior to PDE5-I alone, but further studies are needed to confirm these findings. Investigation on the use of the vardenafil combination is also
very limited. In this regard, the PDE-5I included in this study was limited to tadalafil. In a systematic review by Rim et al., 8.3% of patients treated with L-arginine supplement alone or in combination and 2% of the L-arginine-only group experienced side effects. Studies focusing on the safety aspects of this subject are necessary to strengthen the applicability of the combination therapy further.35,26

The upside of current meta-analysis results is that all outcomes were analyzed from only RCTs. This study is also in line with existing theory and is the first meta-analysis regarding the combination of tadalafil with L-arginine as the treatment for ED. However, a few studies are still discussing the combination of tadalafil with L-arginine as a treatment for ED, as with other PDE5-Is. Concerns regarding the presence of biases in the included studies exist as it could impact the robustness of the outcomes.

Large-scale, multicenter RCT studies investigating the combination of tadalafil or other PDE5-I with L-arginine as the treatment for ED are needed to confirm current perspectives. Future studies also need to evaluate sexual satisfaction in both partners before and after treatment to provide a clear comparison on this subject.

**CONCLUSION**
Combination therapy of tadalafil and L-arginine provide a greater benefit in regard to the increase of IIEF-5 score and testosterone level compared to tadalafil monotherapy in patients with ED.

**CONFLICTS OF INTEREST**
The authors declare that they have no conflict of interest.

**FUNDING SOURCE**
None.

**ETHICS COMMITTEE APPROVAL**
This systematic review and meta-analysis do not require an ethical approval.

**INFORMED CONSENT**
This systematic review and meta-analysis do not need an informed consent statement.

**AUTHOR CONTRIBUTION**
- Donny Austine Wibisono (D.A.W.) is involved in the concept and project design, materials, literature search, data collection and/or processing, analysis and/or interpretation, writing the manuscript, and final approval of the version to be submitted.
- Furqan Hidayatullah (F.H.) is involved in the materials, literature search, data collection and/or processing, analysis and/or interpretation, writing the manuscript, and final approval of the version to be submitted.
- Fikri Rizaldi (F.R.) is involved in the concept and project design, supervision, resources, materials, literature search, data collection and/or processing, analysis and/or interpretation, writing the manuscript, and final approval of the version to be submitted.
- Tarmono Djiojomedjo (T.D.) is involved in the concept and project design, supervision, resources, materials, literature search, data collection and/or processing, analysis and/or interpretation, writing the manuscript, and final approval of the version to be submitted.

**REGISTRATION OF RESEARCH STUDY**
- Name of the registry: PROSPERO
- Unique Identifying number or registration ID: CRD42022343590
- Hyperlink to registration: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=343590

**REFERENCES**