Editorial
The role of social media support as public health intervention strategy in Indonesia ......................... 1

Original Articles
Factors associated to first line antiretroviral therapy (ART) failure among HIV/AIDS patients at Sanglah Hospital, Bali ........................................................................................................ 4
Implementation of quality function deployment to identify priority needs of customers and health providers of child-friendly community health centre ......................................................... 12
Cervical cancer screening among reproductive-aged women: a crosssectional survey in Tabanan Regency .......................................................................................................................... 18
Risk factors of pulmonary tuberculosis among diabetes mellitus patients in Denpasar City .................................................................................................................................................. 24
Barriers for people who inject drug (PWID) to access voluntary counselling and testing (VCT) at the health centres in East Lombok ......................................................................................... 30
High parity and chronic energy deficiency increase risk for low birth weight in Situbondo District ........................................................................................................................................ 35
Evaluation of HIV screening at antenatal care settings in Denpasar City .................................................................................................................................................................................. 35
Risk factors for low birth weight infants in East Nusa Tenggara ......................................................................................... 41
Readiness of girls aged 10-12 years for an early menarche: a transtheoretical model of behavioural change analysis ......................................................................................................................... 49
Association between the use of insecticide-treated bed net and malaria infection in Ende District, East Nusa Tenggara ......................................................................................................... 55
Preparedness of general practitioners in providing health services to foreign tourists in Bali, Indonesia ................................................................................................................................. 62
Delayed access to treatment and frequency of acute respiratory infection as risk factors of severe pneumonia among children aged 12-59 months in Denpasar, Bali ....................................................................... 76
Barriers and opportunities for implementing prevention from mother to child transmission (PMTCT) in Bangli District .................................................................................................................. 83
Sexual behaviours and sexual networks of men who have sex with men in Bali ......................................................................................... 89
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Sexual behaviours and sexual networks of men who have sex with men in Bali
Factors associated to first line antiretroviral therapy (ART) failure among HIV/AIDS patients at Sanglah Hospital, Bali

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Abstract
Background and purpose: The incidence of first line ART failure is increasing in the South East Asia region. The main referral hospital in Bali has recorded an increased use of second line ART due to the first line ART failure. This study aims to explore risk factors associated to first line ART failure.
Methods: A case control study was conducted among people living with HIV and AIDS at Sanglah Hospital Denpasar who started first line ART between 2004 and 2013. Cases were those who diagnosed as having clinical treatment failure and still on treatment in 2015. Controls were those with no treatment failure. Sex and year of ART initiation were matched between case and control. Data were obtained from medical records that include initial regiments, HIV mode of transmission, the WHO HIV clinic al stage, CD4 count, opportunistic infections, body mass index, hemoglobin level, and drug substitution at the beginning and during treatment. Risk factors were analysed using logistic regression.
Results: Out of 68 HIV/AIDS patients with clinical ART failure, 72.1% were confirmed with immunological and 36.8% were confirmed with virological failure. Median time before treatment failure was 3.5 years. Factors associated to ART failure were HIV clinical stage IV with (AOR=3.43; 95%CI=1.65-7.13) and being widow/widower (AOR=4.85; 95%CI=1.52-15.53). Patients with TB co-infection have a lower risk for treatment failure due to early diagnosis and treatment through TB-HIV program with (AOR=0.32; 95%CI=0.14-0.70).
Conclusions: Higher HIV clinical stage at ART initiation increases the risk of treatment failure. HIV-TB co-infection indirectly reduces the risk of treatment failure.
Keywords: treatment failure, first-line ART, HIV/AIDS, Bali

Introduction
People living with HIV and AIDS (PLWHA) require a long-life antiretroviral treatment (ART) to reduce viral load and to prevent infections, drug resistance, complications and AIDS-related deaths.1 Long term ART is associated with lack of treatment adherence which leads to treatment failure and drug resistance.2 The scaled-up of first line ART globally may contribute to the increase of ART failure.3 First line ART failure increases the need for second line ART that are more expensive with higher adverse effects and demand more advance healthcare facilities.4-7 Globally, as many as 14.9 million PLWHA were on ART in 2014 for which 94.8% were on first line ART.8 The average incidence of ART failure in Sub-Saharan African countries is 2.65 per 100 person years, while in the South East Asian countries is only less than 5%.9,10 In Indonesia, from 160,138 PLWHA as many as 97.03% were on first line ART in 2014 however until now the rate of treatment failure is still unknown.

Studies have revealed that ART failure is associated with factors prior to ART initiation
and during the treatment, however these studies are still inconsistent. Factors prior to ART initiation that contribute to ART failure are HIV clinical stage IV and lower CD4 count. During the treatment, lack of adherence and period of treatment contribute to treatment failure. Several other studies have evaluated association between treatment failure and clinical indicators such as opportunistic infections (OIs), drug regimens, modes of HIV transmission, haemoglobin level, body mass index (BMI), drug toxicity and first line ARV resistance. These studies have also examined relationship between treatment failure and sociodemographic variables such as age, sex, education level and marital status.

Data from Bali Provincial Health Office in 2015 showed that as many as 96.2% of 1,173 HIV/AIDS patients were on first line ART. In 2015, there were 54 voluntary counselling and testing (VCT) facilities available across Bali Province, seven hospitals/clinics providing first line ART and only three hospitals providing second line ART. One among those health facilities that is able to provide comprehensive care including ART for HIV/AIDS patients is Sanglah General Hospital. Until 2015, a total of 2,431 HIV/AIDS patients had ever accessed ART from this hospital. In addition, a total of 152 clients were on second line ART or 84% of the total second line ART in Bali Province. Only 68 patients met the case criteria and the rest were excluded due to several reasons that include child (6 cases), allergic (14 cases), unavailability of prior to ART data (20 cases), and incomplete medical record (10 cases). Controls were conveniently selected from 1,831 medical records. Medical records were reviewed against the control selection criteria. Cases and controls were matched for sex and year of initiating ART. This strategy was implemented until 136 controls were selected.

This study defined ART failure based on the standard operational procedure of Sanglah Hospital. Clinical ART failure is confirmed if first line ART has been taken for at least 6 months with observed clinical improvements followed by clinical deterioration such as the present of OIs. Immunological examination (CD4 count) was not regularly performed even though it is a routine procedure. Viral load could not either be routinely conducted due to limited access. Immunologically, ART failure is defined if CD4 count remains the same or reduces up to 50% from initial CD4 count, while virologically if viral load count remains the same or increases more than 5,000 copies/ml from initial viral load count. Risk factors that
examined in this study were sociodemographic and clinical variables. Clinical variables included all variables at ART initiation and during ART. Sociodemographic variables included age, level of education, employment and marital status at ART initiation and during ART. Clinical variables at ART initiation were modes of HIV transmission, first line ART regiments, the most frequent OIs, the WHO HIV clinical stage (stage I-IV), CD4 count, haemoglobin level, and BMI. Clinical variable during first line ART was history of drug substitution due to adverse reactions or side effects. Modes of HIV transmission were defined as potential sources for acquiring HIV that included those with high risk sexual behavior, partner of PLWHA, and injecting drug user. OIs in this study included candidiasis (oral and oro-esophageal), tuberculosis, and hepatitis.

Univariate and bivariate analysis were conducted for each identified risks for ART failure to obtain crude odd ratio. Variables with p<0.25 on bivariate analysis were included in the multivariate analysis. Prior to multivariate analysis, correlation test between variables was performed. If a strong correlation (r>0.6; p<0.05) was found, only one variable will be included in the multivariate analysis. Logistic regression using combination of enter and backward methods was performed to calculate adjusted odd ratio (AOR), p-value and 95%CI.

The study protocol has been approved by Human Research Ethics Committee of Faculty of Medicine Udayana University/Sanglah General Hospital.

Results

Sixty-eight HIV/AIDS patients were confirmed as clinical ART failure with median time of 3.5 years. Forty-nine (72.1%) were confirmed as immunological failure with median time of 3.7 years (95%CI: 2.7-5.1) and 25 (36.8%) were confirmed as virological failure with median time of 4.1 years (95%CI: 3.2-5.1).

Characteristics between cases and controls were comparable for sex (p=1.000), year of initiating ART (p=0.803), religion (p=0.659), place of HIV testing (p=0.178), domicile (p=0.411) and the government insurance ownership (p=0.162).

Table 1. Sociodemographic variables associated to treatment failure at Sanglah Hospital, Denpasar - Bali

<table>
<thead>
<tr>
<th>Variables</th>
<th>Bivariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control n (%)</td>
<td>Cases n (%)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 25 year</td>
<td>15 (11.0)</td>
<td>9 (13.2)</td>
</tr>
<tr>
<td>26-35 year</td>
<td>85 (62.5)</td>
<td>45 (66.2)</td>
</tr>
<tr>
<td>&gt; 35 year</td>
<td>36 (26.5)</td>
<td>14 (20.6)</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>84 (61.8)</td>
<td>39 (57.4)</td>
</tr>
<tr>
<td>Single</td>
<td>46 (33.8)</td>
<td>19 (27.9)</td>
</tr>
<tr>
<td>Widow/widower</td>
<td>6 (4.4)</td>
<td>10 (14.7)</td>
</tr>
<tr>
<td><strong>Education level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>11 (8.1)</td>
<td>10 (14.7)</td>
</tr>
<tr>
<td>Junior-SeniorHigh</td>
<td>110 (80.9)</td>
<td>46 (67.7)</td>
</tr>
<tr>
<td>Primary and under</td>
<td>15 (11.0)</td>
<td>12 (17.7)</td>
</tr>
<tr>
<td><strong>Employment status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>104 (76.5)</td>
<td>49 (72.1)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>32 (23.5)</td>
<td>19 (27.9)</td>
</tr>
</tbody>
</table>

Notes: * = p overall value
Table 1 shows the crude OR of sociodemographic variables that include education level, employment status, age, and marital status with treatment failure. Table 2 shows the crude OR of clinical variables and treatment failure. Bivariate analysis revealed that the HIV clinical stage, candidiasis, CD4 count, tuberculosis, and hepatitis were all eligible for multivariate analysis. Correlation test among these factors also showed a weak correlation.

Multivariate analysis showed that HIV/AIDS patients at clinical stage IV were more likely to experience treatment failure than clinical HIV stage I-III (AOR=3.45; 95%CI: 1.65-7.13). HIV/AIDS patients with treatment failure were more likely to be a widow/widower (AOR=3.69; 95%CI: 1.21-11.27) as can be seen in Table 1. In contrast, HIV/AIDS patients with tuberculosis co-infection prior to first line ART initiation were less likely to experience treatment failure than those without tuberculosis infection (AOR=0.32; 95%CI: 0.14-0.70).

### Table 2. Clinical risk factors associated to treatment failure at Sanglah Hospital, Denpasar - Bali

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control n (%)</th>
<th>Bivariate Cases n (%)</th>
<th>Crude OR</th>
<th>p value</th>
<th>Adjusted OR</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Risk for HIV transmission</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sexual</td>
<td>98 (72.1)</td>
<td>43 (63.2)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLWHA partner</td>
<td>18 (13.2)</td>
<td>12 (17.7)</td>
<td>1.52</td>
<td>0.439</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>IDU</td>
<td>20 (14.7)</td>
<td>13 (19.1)</td>
<td>1.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>First line ART regimen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>95 (69.8)</td>
<td>49 (72.1)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-standard</td>
<td>41 (30.2)</td>
<td>19 (27.9)</td>
<td>0.90</td>
<td>0.745</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Candidiasis (OI)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>59 (43.4)</td>
<td>21 (30.9)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC</td>
<td>47 (34.6)</td>
<td>18 (26.5)</td>
<td>1.08</td>
<td>0.007</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>OEC</td>
<td>30 (22.0)</td>
<td>29 (69.1)</td>
<td>2.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tuberculosis (OI)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>84 (65.4)</td>
<td>53 (77.9)</td>
<td>Ref</td>
<td></td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>47 (34.6)</td>
<td>15 (22.1)</td>
<td>0.54</td>
<td>0.069</td>
<td>0.32</td>
<td>0.14-0.70</td>
</tr>
<tr>
<td><strong>Hepatitis (OI)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>126 (92.7)</td>
<td>58 (85.3)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (7.4)</td>
<td>10 (14.7)</td>
<td>2.17</td>
<td>0.102</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clinical HIV stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I-III</td>
<td>94 (69.1)</td>
<td>34 (50.0)</td>
<td>Ref</td>
<td></td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>42 (30.9)</td>
<td>34 (50.0)</td>
<td>2.24</td>
<td>0.008</td>
<td>3.43</td>
<td>1.65-7.13</td>
</tr>
<tr>
<td><strong>CD4 count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;200</td>
<td>20 (14.7)</td>
<td>4 (5.9)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 200</td>
<td>116 (85.3)</td>
<td>64 (94.1)</td>
<td>2.75</td>
<td>0.075</td>
<td></td>
<td></td>
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<tr>
<td><strong>Hemoglobin level</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>35 (25.7)</td>
<td>14 (20.6)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>101 (74.3)</td>
<td>54 (79.4)</td>
<td>1.34</td>
<td>0.418</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>78 (57.3)</td>
<td>36 (52.9)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>7 (5.2)</td>
<td>5 (7.4)</td>
<td>1.54</td>
<td>0.641</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>51 (37.5)</td>
<td>27 (39.7)</td>
<td>1.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Drugs side effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No substitution</td>
<td>97 (71.3)</td>
<td>44 (64.7)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substitution 1 time</td>
<td>27 (19.9)</td>
<td>20 (29.4)</td>
<td>1.63</td>
<td>0.279</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>Substitution &gt;1time</td>
<td>12 (8.8)</td>
<td>4 (5.9)</td>
<td>0.73</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: * = p overall value
Discussion

This study shows that the diagnosis of treatment failure among HIV/AIDS patients was delayed due to limited access. Late HIV/AIDS clinical stage and being widow/widower increase the risk of treatment failure. In contrast, co-infection with tuberculosis reduces the risk of treatment failure.

Diagnosis of treatment failure in this study is based on clinical criteria. Due to limited testing facilities at Sanglah Hospital, immunologic and virologic tests can only be offered to some patients who can afford it. Therefore the median time from ART initiation to the event of immunological failure (3.7 years) and virological failure (4.1 years) were longer than clinical failure (3.5 years). In contrast, existing studies showed that median time for diagnosing clinical failure is shorter than immunological and virological failures (1.4 to 2.5 years). Among all clinical failure cases in this study, 72.1% underwent immunologic confirmation test while only 36.8% underwent virologic confirmation test. All these patients were at late HIV/AIDS clinical stage (stage IV), with low CD4 count (46 cells/mm$^3$) and high viral load (an average of 296.633 copies/ml). Similar situations have also been found in Malawi, Uganda and Zimbabwe that also use clinical diagnosis to determine treatment failure. Given the fact that Sanglah General Hospital is the referral centre for HIV/AIDS care in Bali Province, governments should support the provision of immunologic and virologic confirmation tests to prevent the delayed diagnosis.

This study also revealed that HIV/AIDS clinical stage IV increases the risk for treatment failure by 3.43 times than clinical stage I-III. Similar findings have also reported by numerous studies in Asia and Africa. As many as 39.3% of HIV/AIDS patients at clinical stage IV in this study were diagnosed with severe OIs that include candidiasis esophageal (77.6%), extra-pulmonary tuberculosis (48.7%), severe anemia (39.3%), hepatitis (19.7%), and toxoplasmosis (9.2%). In addition, they had low CD4 count (23.5 sel/mm$^3$). Previous studies in India and Indonesia reported that patients at HIV/AIDS clinical stage IV presented to hospital with severe OIs, low CD4 count and high viral load. Studies in Asia and Africa also showed that patients at late HIV/AIDS clinical stage often presented to health facilities with severe OIs and low CD4 count. Due to these clinical conditions, they required a longer recovery period and a high adherence rate. Therefore they tended to have higher risk of developing treatment failure.

HIV/AIDS patients co-infected with tuberculosis seem to have a lower risk of treatment failure. The implementation of TB-HIV collaboration leads to an early HIV diagnosis among tuberculosis patients followed by an early ART initiation. HIV-TB co-infection patients receive monitoring from Directly Observed Treatment Short Course (DOTS) program as well as from VCT program – leading to better adherence towards HIV and TB treatments. From all TB-HIV co-infection cases in this study, 40.3% HIV status were confirmed after the TB diagnosis. The majority of these patients (82.3%) received ART at four weeks after the commencement of TB treatment. DOTS program facilitates treatment compliance for both HIV and TB. Studies in Kenya and South Africa have also revealed that TB-HIV collaboration improves compliance towards ART and reduces drop out of TB medication. However, previous studies in West Java and South Africa found that HIV-TB co-infection was not associated with treatment failure.

Being widow/widower increases the risk of treatment failure when compared to married HIV/AIDS patients. This finding is consistent with study conducted in Brazil, however the proportion of widow/widower in the present study was small (only 7.8% from...
total sample). Therefore, this finding should be interpreted with caution.

This study has several limitations. Primary source of data in this study is medical record that is often incomplete and confirmation from other sources is required. This may influence the internal validity of this study. Several key factors associated to treatment failure are unavailable for example data on compliance rate to ART. In this study, drugs could also be taken not directly by patients thus making it difficult to accurately measure patients’ compliance. HIV patients only present to the clinic if they experience symptoms associated to treatment failure. Since controls were selected using convenience method, it may not accurately represent the control population.

Conclusion

Clinical diagnosis of treatment failure is delayed. This delay leads to deterioration of patients’ clinical conditions. In addition, severe clinical conditions can increase the risk of first line ART failure. TB-HIV co-infection reduces the risk of treatment failure due to early diagnosis and treatment. Monitoring of viral load is essential to prevent ART failure or CD4 count monitoring if viral load test is not available.

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10 | July 2017 | Volume 5 | Issue 1 |


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1. Manuscript must be written in English or Bahasa Indonesia of maximum of 3000 words (not includes abstract) and consists of 3-4 key arguments, and 3-4 tables and or graphs. It must be written in Microsoft Word with the maximum file capacity of 5 MB. Manuscript must be electronically submitted. Editors can change the format of the manuscripts but not the content.
2. Title must be concise and ensure it reflects the subject matter. Title page should no longer than 18 words.
3. Authors’ name and affiliation must be placed under the title. Corresponding author’s email address must be stated to allow further discussion and interaction with the audience.
4. Abstract should no longer than 300 words and must reflect the subject matter which includes: background and purpose, methods, results, and conclusion. It should also be accompanied by 3-5 key words.
5. Introduction must concisely address the existing gaps in the literature and state precisely study objectives.
6. Methods must clearly outline the study design, population, sample, source of data, data collection techniques, research instruments, and data analysis.
7. Results present findings of the study without opinion of the authors. Findings should be concise and can be presented using tables, graphs, and narratives. Table must be single space and must be numbered based on its occurrence in the text. The maximum of four tables and/or graphs are allowed which must contain a short self explanatory title. The title of table is place above the table with left alignment, single space. The title of graph is place under the graph with centre alignment, single space.
8. Discussion explains precisely findings of the study supported by sound theoretical and evidence from previous studies. Specific to qualitative studies, findings can be presented alongwith the discussion.
9. Conclusion should answer the research questions and can include a brief recommendation.
10. Acknowledgements should be addressed to related stakeholders who had supported the study, including respondents.
11. Reference lists
   It contains all references cited in the text. Referencing format must follow the Vancouver style (superscript without bracket), and should refer to the most up-to-date available evidence. Author’s last name followed by the initials of their first and middle name should be consistently used. When the authors are up to six, all authors should be written, but when those are more than six, the first six authors should be written followed by et al. The title of article must be written in sentence case. If the journal acronym is used, it should confirm to Medicus Index. Examples of referencing styles of different sources can be seen in the appendix.
12. Authors should pay attention on their writing structure, including sentence structure, accuracy of the text, table or graph. All accepted manuscripts will be provided back to the authors if the format has not complied with the instruction guidelines.
13. Authors must state their full name, qualifications, corresponding address, and affiliations. They should also complete the agreement form of right transfer for publication purposes only.
14. All manuscripts subject to peer review processes and reviewed by editors. Further revision is requested prior to publication, or rejected for publication. Editors will provide the final decision and notify the authors whether the manuscript is accepted for publication.
15. Accepted manuscript written in Bahasa Indonesia will be translated by the PHPMA production editor, with the cost of IDR 3,000,000.
16. Manuscript must be submitted electronically to the following email: jurnalmikm@gmail.com
Appendix 1. Referencing guidelines

Every cited reference must appear in the reference lists and vice versa. The citation in the text should be numbered, for example: 1 or 2. If the citation is more than two references, only the first and the last number are written separated by ‘dash’, for example 1-3 or 3-8. The citation must be superscript and must be placed after the text, for example: Nutritional assessments can be done by several methods which are anthropometric\(^1\), dietetic\(^2\), and biochemistry tests.\(^3\)

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**Example:**

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**Example:**

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**Example:**

**Editors, compilers as the authors**

**Example:**

**Organisation as the author and publisher**

**Example:**

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**Example:**

**Conference paper**

**Example:**

**Scientific or technical report**

**Example:**

**Thesis (PhD, Master or Undergraduate)**

**Example:**

**Electronic journal article**

**Example:**
Appendix 2. Guidance for statistical reporting

This guidance is provided to assist authors preparing for their statistical report for publication. This guidance is not to replace the existing statistical guidelines required in a quantitative study. Each component is elaborated below.

Abstract:
Total sample and source of data must be clearly stated. Any conclusion made from statistical tests must be accompanied by descriptive statistic reports for example mean, median, mode, standard deviation, interquartile, variation coefficient percentage, 95% confidence interval, regression formula, and so forth.

Methods:
For an experimental study, sampling technique and randomisation procedure must be clearly provided. If applicable, analytical precision should also be stated. Statistical hypothesis must be clearly stated. Power of the study should be provided in relation to sample size calculation (it is recommended to use at least 80%). For a case control design, selection procedures for cases and controls must be explained in great depth. When applicable, matching procedure should also be clearly stated. For a diagnostic study or a clinical trial, it is recommended to refer to other reporting structures for example STARD, CONCORT, or STROBE.

Results:
Any insignificant precision should be avoided, especially when presenting data using table. A rounded data is easier to read and often decimal numbers are not essential. It is recommended for percentage data to report only one decimal digit (for example 27.9%). If the sample size is relatively small, it is strongly recommended to avoid decimal numbers. Data distribution must be reported in terms of mean, standard deviation, or coefficient variation percentage and must be reported as ‘mean (SD)’ instead of ‘mean ± SD’. If data are not normally distributed (after the Shapiro Wilk Test), median and interquartile range must be used to replace mean and standard deviation. A skewed data could be normalised by applying a logarithm or power transformation. All statistical analysis must used this transformed data which then must be re-transformed for data presentation. All individual values must be presented (if applicable) by deleting all overlapping values. Error bars which reflect standard error for each mean value or interquartile range for each median value can be used to guide data interpretation. Each statistical test such as chi square test must be reported with the descriptive data, degree of freedom and p-value. Validity of each assumption prior to the test should be examined (for example data should be normally distributed when a t-test is used with the same variance for each data set). When a contingency table is used (2x2 table) for chi square test, continuity correction should be considered and if the expected count is low, the Fisher Exact value should be used. P-values should be clearly provided to show significance of such test. When the statistical test shows a very significant result and p-value from the computer program calculation is 0.0000, p-value should be presented as ‘p<0.0005’. Confidence interval must also be clearly stated, particularly for the insignificant results. As a general principle, statistical analysis should be reported as p ≤ 0.05. If another method is used, this must be clearly justified on the method section of statistical analysis.

Discussion:
A result of statistical test is not the most critical point of discussion. It is recommended that p-value should not be compared for different data set or for a different statistical analysis. Each association must not be interpreted as causal relationship without a sound supporting evidence.
Statistical issues:

Multiple Comparisons
This can cause misleading interpretation for significance values. Primary hypothesis must be clearly stated. Every association identified from a retrospective method must be interpreted with cautions. If applicable, one statistical test should be performed to all variables, for example ANOVA test. If this test is not significant, multiple comparisons thus can be applied. If ANOVA test is not applicable (or related statistical tests), multiple comparisons can be applied by referring to Bonferroni test.

Paired Data
For paired data, the difference for each pair and variability from these differences is more significant than the values of each individual. It is recommended to use graph for example plotted lines to present paired data.

Standard regression analysis
To perform this analysis, independent data are required (repeated measurements are not an independent data). Independent variables are measured without significant errors and all data must be normally distributed without outliers. These can be easily tested using a scatter plot diagram.

Method comparison
It is inappropriate to compare methods using regression and correlation coefficient. It is recommended to use the Altman and Bland Difference Plot. If regression and standard scatter plot are considered useful, it can be presented along with the Altman-Bland Plot. It should always be considered that if two methods are supposed to measure the same matter, it is highly possible that both are correlated, therefore correlation value provides limited information for interpretation. When a more complex statistical analysis is performed for example a multivariate analysis including ROC test or other tests, it is recommended that the authors should consult to statisticians.
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