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Body mass index variation over time and associated factors among HIV positive adults on second line ART in Northwest Ethiopia: A retrospective follow up study

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3 1 **Body mass index variation over time and associated factors among**
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6 2 **HIV positive adults on second line ART in Northwest Ethiopia: A**
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9 3 **retrospective follow up study**
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19 Abstract

20 **Objectives:** This study aimed to assess the evolution of Body Mass Index of HIV positive adults
21 on second line ART over time and factors affecting it in Northwest Ethiopia.

22 **Design:** Institution based retrospective follow up study was conducted using data extracted from
23 1016 patient cards from February 2008 to February 2016.

24 **Setting:** Eight referral hospitals from Amhara region, Ethiopia were included

25 **Participants:** HIV patients who started second line antiretroviral therapy

26 **Outcome measures:** Change in BMI since starting second line antiretroviral therapy

27 **Results:** Five hundred thirty eight (52.95%) of participants were males and median age of
28 participants was 33 (P25=28, P75=39). The median follow up time was 18 months (P25=5.2,
29 P75, 32.2 months). The average change of BMI has showed a linear increase over time. The
30 amount of BMI increment or decrement according to each variable was shown as β coefficients.
31 Treatment duration ($\beta=0.013$, 95% CI (0.004, 0.022)), Isoniazid prophylaxis ($\beta=0.87$, 95% CI
32 (0.32, 1.42)), Cotrimoxazole prophylaxis ($\beta=0.63$, 95% CI (0.08, 1.19)), ambulatory functional
33 status($\beta=-1.16$, 95% CI (-1.95, -1.31)), bedridden functional status($\beta= -1.83$, 95% CI (-2.47, -
34 1.21)), WHO stage III($\beta=-0.42$, 95% CI (-0.65, -0.20)) WHO stage IV ($\beta= -0.62$, 95% CI (-1.02,
35 -0.22)), CD4 count ($\beta=0.001$, 95% CI (0.0008, 0.0015)); and time interaction of variables like
36 tertiary educational status($\beta=0.02$, 95% CI (0.01, 0.04)), ambulatory functional status($\beta=0.03$,
37 95% CI (0.01, 0.05)) and WHO stages III ($\beta=0.01$, 95% CI (0.007, 0.02)) were found to be
38 significant predictors.

39 **Conclusion:** BMI of patients has shown a linear increment over the treatment time. Factors
40 affecting it have been identified but its effect on Cardio-vascular disease needs further study.

41

Strengths and limitations of this study

- Being a retrospective study, this study shares the limitations of secondary data collection as a result we were unable to find some predictors like viral load, alcohol consumption, smoking and nutritional history.
- The study is not based only on patient cards that started the second line antiretroviral therapy at the same point in time and also the length of follow up period for each participant was not equal across all participants
- The most important strength of this study was the use of longitudinal data analysis. This ensures a valid estimate by handling data that is measured in different time period and has missing values.
- Besides we had a total of 5380 number of BMI measurements taken from 1016 participants with a median BMI of 20.52 Kg/m² (P25= 18.2, P75=23). Baseline BMI was calculated for all 1016 patients. These will again strength the precision of the estimates.

64 **Background**

65 Human Immunodeficiency Virus (HIV) has continued to be a major global public health
66 problem, having killed more than 35 million lives so far. In 2017, 940 000 people died from
67 HIV-related causes globally(1). According to the Ethiopia demographic and health survey of
68 2016 the adult prevalence of HIV in Ethiopia was 0.9%(2). Death due to HIV/AIDS has
69 enormously decreased due to the introduction of anti retroviral therapy (ART) and millions of
70 people are currently accessing it (3).

71 Body weight of HIV patients has been an important diagnostic and evaluation measure. Low
72 BMI is recognised as one of the first criteria for the clinical definition of Acquired Immune
73 Deficiency Syndrome (AIDS). The current World Health Organization (WHO) clinical staging
74 of the disease also includes moderate unexplained weight loss (<10% presumed or measured
75 body weight, stage 2), unexplained severe weight loss (>10% of body weight or BMI \leq 18.5
76 kg/m², clinical stage 3) and HIV wasting syndrome (Unexplained severe wasting, clinical stage
77 4) as criteria to define advanced HIV infection(4).

78 The BMI of HIV patients is an important predictor of ART treatment outcome (5-8), including
79 the prediction of the CD4 cells change (9) and death (10-12). Negative change in BMI of patients
80 was also found to be an independent predictor of dropout from HIV care (13).

81 The BMI of HIV patients is affected by gender(14) duration of treatment (10, 15, 16), Isoniazid
82 Prophylaxis Therapy (IPT) and Cotrimoxazole prophylaxis(CPT) (17, 18) WHO stage (14) and
83 CD4 count (15, 19).

84 Even though BMI evolution is a very important and easily calculable tool that can predict
85 treatment outcome, dropout from treatment, CD4 recovery and death literatures about its

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3 86 evolution across treatment time and factors affecting it are limited. Therefore this study tried to
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5 87 fill this information gap by determining the BMI evolution over treatment time and identifying
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8 88 the key factors affecting it. Information generated by this study will contribute to monitor the
9
10 89 response of patients for antiretroviral therapy.

11 12 90 **Method and materials**

13 14 15 91 **Study design and period**

16
17 92 An institution based retrospective follow up study was conducted among adults, age 24 and
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19
20 93 above who are started on second line ART from February 2008 to February 2016

21 22 94 **Study area and population**

23
24 95 The study was conducted in Amhara regional state which is one of the nine administrative
25
26
27 96 regions and two city councils of Ethiopia. The region constitutes majority of ART users in the
28
29 97 country. The study population was adult HIV infected patients on second line ART in all the nine
30
31 98 referral hospitals of the region. The first-line treatment consists of a combination of two
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33
34 99 nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) with one non-nucleoside reverse
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36 100 transcriptase inhibitor (NNRTI). Should failure of first-line treatment occur, a second-line
37
38 101 treatment is implemented, using two NRTIs not previously used in first-line treatment, as well as
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40
41 102 one additional protease inhibitor (PI).

42 43 103 **Sample size and sampling procedure**

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45 104 All HIV patients who initiated second line therapy (n=1233) between February 2008 and 2016
46
47 105 were included. But only 1016 patients who had two or more measurements of weight were
48
49
50 106 included in the study.

107 **Data collection procedures**

108 Data extraction check list was prepared and the data were collected from HIV patient registration
109 cards. Data about the baseline weight and height of patients and other factors like socio-
110 demographic, clinical, and treatment related factors were also collected from each HIV patient
111 registration cards. Adherence to ART was assessed by pill counts at visits and is recorded as
112 ‘GOOD’ ($\geq 95\%$ adherence) or FAIR (80%–95%), while POOR adherence is less than 80% (20).

113 **Data structure, compilation and analysis strategy**

114 The data was cleansed and entered in to EPI info version 7 and analyzed using STATA version
115 14.0. Body Mass Index (BMI) of HIV patients was computed by dividing the weight of patients
116 in kilogram (kg) that was recorded during each follow up visit, to their baseline height in meter
117 squared. Exploratory data analysis for the weight of patients including individual profile plot,
118 mean profile plot, descriptive and summary statistics were done. To determine the factors
119 associated with BMI of patients univariate analysis for each independent variable was assessed
120 and those found to be significant ($p\text{-value} < 0.25$) were selected for the multivariate analysis. The
121 need for random intercept and random slope was checked by likelihood ratio test. Finally a
122 model that can handle repeated measurements that have between individual variation and within
123 individual variation, that is linear mixed effect model with random intercept and random slop
124 was fitted.

125 **Patient and Public Involvement**

126 Since we have used secondary data/chart review, patients or the public were not involved during
127 identifying the research question or design and conduct of the study.

128

129 **Results**

130 **Patient characteristics**

131 We had a total of 1016 adult patients who were taking second line ART. Among the total 538
132 (52.95%) were males. All participants were above or equal to the age of 24 with median age of
133 33 year (P25=28, P75=39). Large proportion of these patients 370 (37.1%) were unemployed
134 and 371 (36.9%) have attended secondary school.

135 The median follow up time was 18 months (P25=5.2, P75, 32.2). There were a maximum of 12
136 and a minimum of 2 measurements of weight per patient. Almost all visits were not balanced in
137 their time of measurements. The median time between each weight measurement was
138 6.7(P25=4.27, P75=10.03), 6.08(P25=4.14, P75=9), 6.13(P25=4.2, P75=8.9), 6.27(P25=4.53,
139 P75=8.9), 5.9(P25=3.97, P75=7.79), 5.83(P25=3.7, P75=8.13), 5.45 (P25=3.37, P75=7.43),
140 4.86(P25=2.97, P75=6.2), 5.19(P25=3, P75=7.37), 5.97(P25=4.23, P75=7.04), 5.55(P25=2.83,
141 P75=5.86) and 3(P25=0.9, P75=4.84) months from first to twelfth weight measurement
142 respectively.

143 At the initiation of second line ART majority of participants, 393 (45.54%), were at WHO stage
144 I and 324 (37.54%) were at WHO stage III. Large proportion of participants 871 (86%) had
145 working functional status at base line, the rest 114 (11.25%) and 28 (2.75%) were ambulatory
146 and bed ridden respectively. The median CD4 count was 253 (P25=147, P75=399). Isoniazid
147 Prophylaxies Therapy and CPT were given for 247 (24.87%) and 256 (25.65%) of participants
148 respectively (**Table 1**).

149 **Exploratory data analysis**

150 At baseline a total of 391(38.5%) had BMI of less than 18.5kg/m² whereas 549(54%), 64(6.3%)
151 and 12(1.2%) had BMI 18.5-24.9 kg/m², 25-29.9 kg/m² and \geq 30kg/m² respectively.

152 **Individual Profile**

153 The average BMI of patients at baseline was 19.03Kg/m² (SD=3.6 Kg/m²). The minimum and
154 maximum BMI was 8.33 Kg/m² and 33.59 Kg/m² respectively. Before proceeding to the formal
155 statistical analysis we have described the data by exploring how individuals change in BMI over
156 time. To make the individual profile plot more informative and understandable, we have
157 prepared it for the first 100 individuals (**Figure 1**).

158 As we can see from this plot, the BMI status of patients has high variability within an individual
159 over time and among individuals at baseline and also through time. Therefore to fit the data
160 which has variability in the intercept and slope of trajectories very well, we have used a mixed
161 model.

162 **Exploring the mean profile**

163 As stated above all measurements in the data have no similar time of measurements therefore we
164 have used smoothing to determine BMI evolution over time (**Figure-2**).

165 As one can easily understand from the mean profile plot the BMI of these patients showed a
166 linear increment over the treatment time.

167 **Modeling the BMI**

168 **Correlation structure checking and model comparison**

169 Comparing with AIC, the correlation structure with lowest AIC was chosen, in this case the
170 unstructured correlation structure (**Table-2**).

171 The need for random slop and intercept was checked by likelihood ratio test of model without a
172 random intercept, with only random intercept and a model with both random intercept and slop
173 (**Table-3**).

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3 174 So as we can see from the table-3 the inclusion of random intercept and random slop is
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5 175 reasonable so in the final model we have used both random intercept and random slop.
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8 9 176 **Factors affecting the BMI evolution over time**

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11 177 Based on the multi-variable output: time since the start of second line ART, IPT , CPT,
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13 178 Functional status, WHO stage of the disease; and time interaction of categorical variables like
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15 179 educational status, functional status and WHO stages were found to be significant predictors of
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17 180 the BMI evolution of HIV patients on second line ART.

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19 181 Keeping all the other variables constant, for a one month increment in the treatment duration the
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21 182 BMI of patients' increases by 0.013 kg/m². But specifically individuals who have tertiary level of
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23 183 education, ambulatory functional status and who are at WHO stage III have additional 0.02
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25 184 kg/m², 0.03 kg/m² and 0.01 kg/m² increment respectively in their BMI for a one month
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27 185 increment in the duration of treatment.
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31 186 Taking IPT and CPT increases BMI by 0.87 kg/m² and 0.63 kg/m² respectively as compared to
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33 187 their counter parts. Nongovernmental organizations employment was associated with a 2.02
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35 188 kg/m² increment when compared to government employment. Patients who are ambulatory and
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37 189 bedridden in their functional status have a 1.16 kg/m² and 1.83 kg/m² decrement in their BMI as
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39 190 compared to those who have working functional status. Patients who are at WHO stage III and
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41 191 WHO stage IV have decreased BMI by 0.42 kg/m² and 0.62 kg/m² when compared with those
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43 192 who are at WHO stage I. For a unit cell/mm³ increase in the CD4 count of patients BMI was
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45 193 found to increase by a factor of 0.001 kg/m² (**Table-4**).
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48 49 194 **DISCUSSION**

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53 195 This study has found a linear increment in BMI of patients over treatment time. There were BMI
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55 196 differences between subjects at baseline and in their progress over time.
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3 197 Factors found to have a significant effect on the evolution of BMI over treatment time at
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5 198 multivariable linear mixed effect model were second line treatment duration, IPT, CPT,
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7 199 employment status, functional status of the patient, WHO stage and CD4 count. Time interaction
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10 200 of educational status, functional status, and WHO stage.

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12 201 For a one month increase in treatment duration the BMI of patients increases by 0.04, this
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14 202 finding is in line with studies in South Africa (21), India (10) and United states(22). The possible
15
16 203 reasons for weight gain could be due to normal reversion of the weight loss associated with HIV
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18 204 or due to drug related metabolic changes which include hyperlipidemia, insulin resistance and
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20 205 diabetes(23).

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22 206 Patients who took IPT and CPT have increased BMI when compared to their counter parts. This
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24 207 finding is supported by other studies like a study conducted in Abidjan (17) and multicenter
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26 208 controlled clinical trial in Africa (18). The main reason for this association could be the reduction
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28 209 of potentially disabling and wasting disease like tuberculosis and other opportunistic infections
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30 210 by these prophylactic drugs(24, 25). A highly increased appetite by the prophylaxis could also be
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32 211 possible explanation(26).

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34 212 Being ambulatory and bedridden decreases BMI when compared to those who are working.
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36 213 Since these individuals are not working they may not access nutritious and balanced diet which
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38 214 affects their BMI. Another reason could be additional decreased immunity caused by physical
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40 215 inactivity which makes them more susceptible to minor infections leading to a higher calorie
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42 216 loss(27). These group of people are also at higher risk of diarrheal disease for they cannot take
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44 217 care of themselves which in turn causes weight loss(28).

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46 218 When compared with patients who are at WHO stage I, those who are at WHO stage III and
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48 219 WHO stage IV have decreased BMI. This finding is in line with a multicenter study in resource
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3 220 limited settings which shows individuals who had high clinical status (WHO stage III and WHO
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5 221 stage IV) had poorer weight gain when compared to weight change in patients at lower WHO
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7 222 stage(14). The possible reason can be unexplained chronic diarrhea and HIV enteropathy in these
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9 223 patients and the associated malabsorption (4) or it could be due to pyrexia of unknown origin in
10
11 224 the late stage of the disease which results an increased calori loss and wasting(29).

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13
14 225 The BMI of patients was also found to increase with the increment in their CD4 count. This
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16 226 positive association is supported by evidence from a study conducted in Boston (15) and
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18 227 Tanzania (19). This increment can be explained by association of increment of CD4 count with
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20 228 good clinical changes like viral suppression, improved immunity and appetite leading to
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22 229 increment in the BMI of patients (13).

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25 230 Being a retrospective study, this study shares the limitations of secondary data collection as a
26
27 231 result we were unable to find some predictors like viral load, alcohol and smoking marital status
28
29 232 and nutritional history.

30 31 32 33 34 233 **CONCLUSION**

35
36 234 In this study we have found a linear increment in the BMI of HIV patients on second line ART.
37
38 235 There was a significant variation of BMI of patients at baseline and through ART treatment time.
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40 236 Duration of treatment, IPT, CPT, functional status, WHO stage of the disease, CD4 count; and
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42 237 time interaction of categorical variables like educational status, functional status and WHO
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44 238 stages were found to be significant predictors. The positive change in the BMI of patients shows
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46 239 an encouraging trend for we know this has a positive impact on the CD4 recovery, decrease lost
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48 240 to follow up and death. Clinicians also must consider the identified risk factors when they
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50 241 provide service for these patients.

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243 **ABBREVIATIONS**

244 AIC, Akaike's Information Criterion; AIDS, Acquired Immunodeficiency Virus; ART,
245 Anti-Retroviral Therapy; BIC, Bayesian information criterion; BMI,
246 Body Mass Index; CI, Confidence Interval; CPT, Co-trimoxazole preventive therapy; HIV,
247 Human Immuno Deficiency Virus; IPT, Isoniazid Prophylaxis Therapy; Kg,
248 Kilogram; NGO, Non Governmental Organizations; WHO,
249 World Health Organization.

250 **Declarations**

251 **Ethical consideration**

252 Ethical clearance was obtained from Institutional Review Board of Institute of Public Health,
253 University of Gondar. Names and unique ART numbers of patients was not collected to keep the
254 privacy of patients during the data collection.

255 **Availability of data and materials**

256 The data upon which the result based could be accessed by a reasonable request made to the
257 corresponding author.

258 **Funding**

259 Not applicable

260 **Consent for publication**

261 Not applicable

262 **Competing interest**

263 Authors declare that they have no any conflict of interest

264 **Authors' contribution**

1
2
3 265 AGB, LDG, EGZ, TAA, ATT have actively participated during Conception and design,
4
5 266 acquisition of data, analysis and interpretation of data. All authors have read and approved the
6
7
8 267 final version of the manuscript.
9

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13
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15
16
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19

20 272 **Authors' information**

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22 273 Adhanom Gebreegziabher, has BSc degree in Public health as a background and second degree
23
24 274 in Epidemiology and Biostatistics; now teaching Epidemiology, Research methodology and
25
26
27 275 communicable disease control courses in the University of Gondar, Ethiopia
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4 371 **Figures Legend**

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7 372 **Figure 1: Individual profile plot of BMI over time for the first 100 individuals at second**
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9 373 **line ART in Amhara region, 2008-2016**

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12 374 **Figure 2: Time plot of BMI versus treatment time in months with lowess smoothed curve**
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15 375 **superimposed for HIV patients on second line ART in Amhara region 2008-2016**

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386 **Tables**

387 Table 1 Baseline socio-demographic and baseline characteristics of adults HIV patients on second
 388 line ART in Amhara region, 2008 to 2016

Variables	Number	Percentage
Age		
25-34	547	53.89
35-44	333	32.81
45-54	104	10.25
55-64	26	2.56
≥ 65	6	0.59
Sex		
Female	478	47.05
Male	538	52.95
Educational status		
Illiterates	315	31.34
Primary education	201	20.00
Secondary education	371	36.92
Tertiary education	118	11.74
Missing	11	0.01
Occupation		
Unemployed	370	36.42
Governmental	286	28.15
Non-Governmental	21	2.06
Private	66	6.50
Daily laborers	254	25.00
Missing	19	1.87
WHO stages		
Stage I	393	45.54
Stage II	66	7.65
Stage III	324	37.54
Stage IV	80	9.27
Functional status		
Working	871	86
Ambulatory	114	11.25
Bed ridden	28	2.75
CPT given		
No	742	74.35
Yes	256	25.65
INH given		
No	746	75.13
Yes	247	24.87

389 CPT= Cotrimoxazole Prophylaxis Therapy

390 INH= Isoniazide

391 **Table 2: Correlation structure checking and model comparison**

	Unstructured	Identity	Exchangeable
AIC	14337.93	17775.34	17666.17
BIC	14531.08	17956.02	17853.09

393 **Table 3: Random Effects Models comparison**

Random effects	LR X ²	P value
Model1 intercept	5625.28	0.0000
Model2 intercept, time	297.13	0.0000

405 **Table 5 Parameter estimates for full linear mixed effect model**

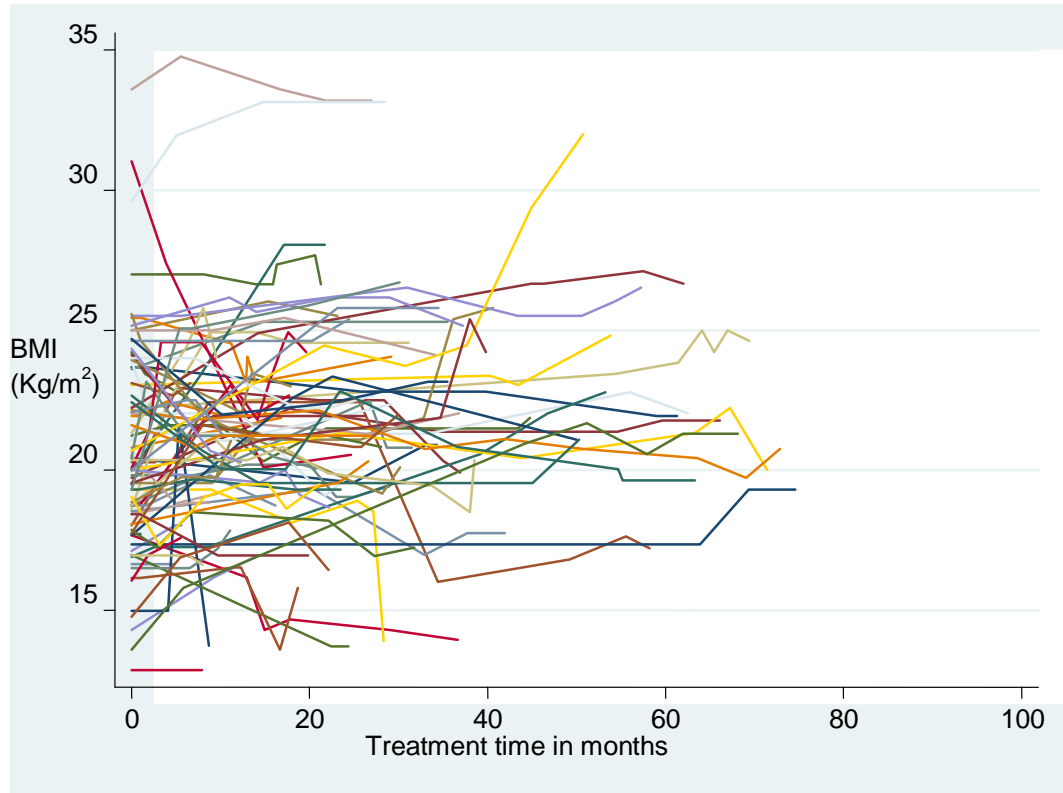
Characteristics	Co-efficient	Confidence interval		P value
		Lower	Upper	
Intercept	19.03	18.11	19.95	0.000
Sex				
Female	0.00			
Male	-0.01	-0.51	0.48	0.956
Adherence				
Poor	0.00			
Fair	0.43	-0.24	1.10	0.207
Good	0.48	-0.06	1.02	0.080
INH prophylaxis given				
No	0.00			
Yes	0.87	0.32	1.42	0.002
CPT prophylaxis given				
No	0.00			
Yes	0.63	0.08	1.19	0.025
Educational status				
Illiterate	0.00			
Primary	-0.03	-0.72	0.66	0.924
Secondary	0.47	-0.21	1.14	0.176
Tertiary	0.94	-0.01	1.89	0.051
Functional status				
Working	0.00			
Ambulatory	-1.16	-1.95	-1.31	0.000
Bedridden	-1.83	-2.47	-1.21	0.000
WHO stage				
Stage I	0.00			
Stage II	-0.06	-0.45	0.33	0.760
Stage III	-0.42	-0.65	-0.20	0.000
Stage IV	-0.62	-1.02	-0.22	0.002
CD4 count	0.001	0.0008	0.0015	0.000
Time on treatment	0.013	0.004	0.022	0.005
Education status x time				
Illiterate x time	0.00			
Primary x time	0.002	-0.01	0.015	0.801
Secondary x time	0.01	-0.003	0.019	0.162
Tertiary x time	0.02	0.01	0.04	0.009
Functional status x time				
Working x time	0.00			
Ambulatory x time	0.03	0.01	0.05	0.000
Bedridden x time	-0.01	-0.05	0.02	0.49
WHO stage x time				
Stage I x time	0.00			
Stage II x time	0.004	-0.008	0.166	0.522
Stage III x time	0.01	0.007	0.020	0.000
Stage IV x time	-0.0003	-0.016	0.016	0.969

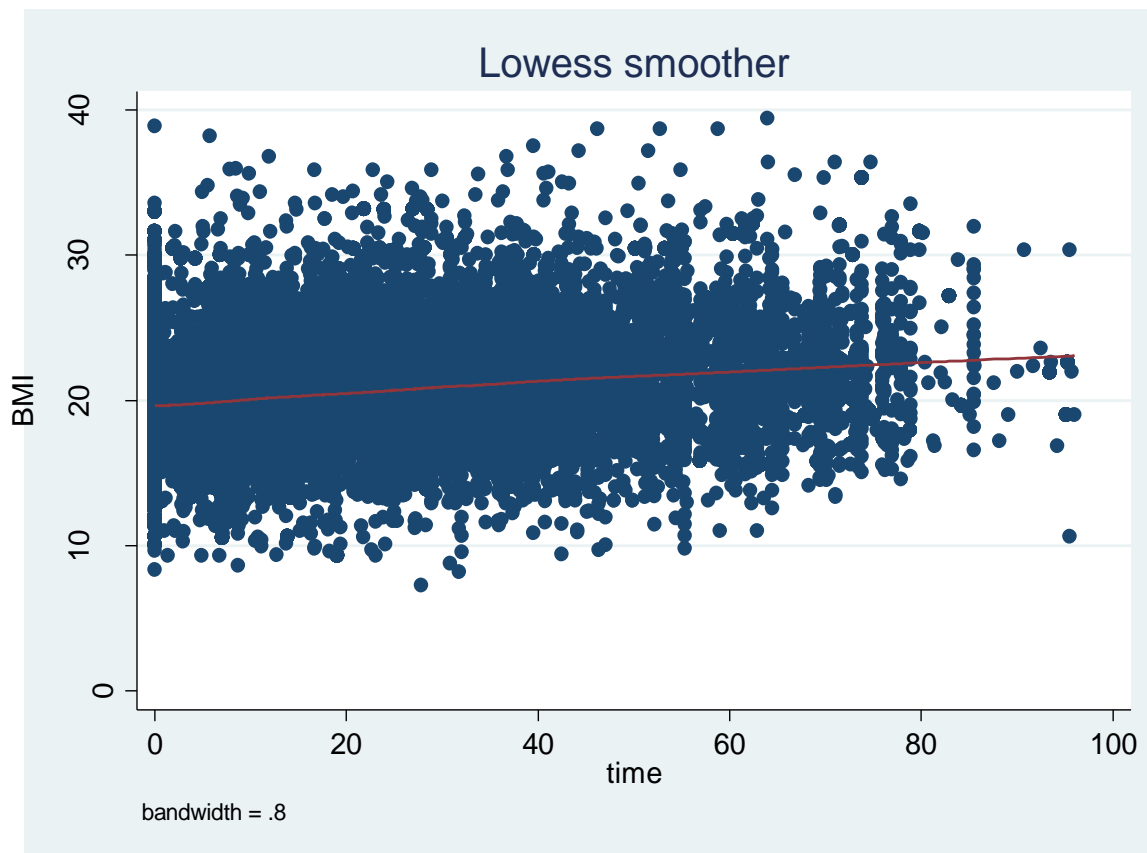
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For peer review only

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Review only

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (b) For matched studies, give matching criteria and number of exposed and unexposed	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) If applicable, explain how loss to follow-up was addressed (e) Describe any sensitivity analyses	6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	Report numbers of outcome events or summary measures over time	7-9

1	Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-9
2			(b) Report category boundaries when continuous variables were categorized	
3			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
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9	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	--
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11	Discussion			
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13	Key results	18	Summarise key results with reference to study objectives	9-10
14	Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
15				
16	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10 - 11
17				
18	Generalisability	21	Discuss the generalisability (external validity) of the study results	11
19				
20	Other information			
21				
22	Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12
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*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.

BMJ Open

Body mass index variation over time and associated factors among HIV positive adults on second-line ART in Northwest Ethiopia: A retrospective follow up study

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Primary Subject Heading:	Public health
Secondary Subject Heading:	HIV/AIDS
Keywords:	Body mass index, Second line ART, Linear mixed effect model

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Manuscripts

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4 1 **Body mass index variation over time and associated factors among**
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7 2 **HIV positive adults on Second-line ART in Northwest Ethiopia: A**
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15 5 Adhanom Gebreegziabher Baraki*, Lemma Derseh Gezie, Ejigu Gebeye Zeleke, Tadesse Awoke
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19 Abstract

20 **Objectives:** This study aimed to assess the evolution of Body Mass Index of HIV positive adults
21 on second line ART over time and factors affecting it in Northwest Ethiopia.

22 **Design:** Institution based retrospective follow up study was conducted using data extracted from
23 1016 patient cards from February 2008 to February 2016.

24 **Setting:** Eight referral hospitals from Amhara region, Ethiopia were included

25 **Participants:** HIV patients who started second-line antiretroviral therapy

26 **Outcome measures:** Change in BMI since starting second-line antiretroviral therapy

27 **Results:** Five hundred thirty eight (52.95%) of participants were males and median age of
28 participants was 33 (IQR: 28; 39). The median follow up time was 18 months (IQR: 5.2; 32.2).

29 The average change of BMI has showed a linear increase over time. The amount of BMI
30 increment or decrement according to each variable was shown as β coefficients. Treatment
31 duration ($\beta=0.013$, 95% CI (0.004, 0.022)), Isoniazid prophylaxis ($\beta=0.87$, 95% CI (0.32, 1.42)),
32 Cotrimoxazole prophylaxis ($\beta=0.63$, 95% CI (0.08, 1.19)), ambulatory functional status ($\beta= -$
33 1.16, 95% CI (-1.95, -1.31)), bedridden functional status($\beta= -1.83$, 95% CI (-2.47, -1.21)), WHO
34 stage III ($\beta=-0.42$, 95% CI (-0.65, -0.20)) WHO stage IV ($\beta= -0.62$, 95% CI (-1.02, -0.22)), CD4
35 count ($\beta=0.001$, 95% CI (0.0008, 0.0015)); and time interaction of variables like tertiary
36 educational status ($\beta=0.02$, 95% CI (0.01, 0.04)), ambulatory functional status ($\beta=0.03$, 95% CI
37 (0.01, 0.05)) and WHO stages III ($\beta=0.01$, 95% CI (0.007, 0.02)) were found to be significant
38 predictors.

39 **Conclusion:** BMI of patients has shown a linear increment over the treatment time. Factors
40 affecting it have been identified but its effect on Cardio-vascular disease needs further study.

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56 43 **Strengths and limitations of this study**
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- 8 44 • Being a retrospective study, this study shares the limitations of secondary data collection,
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10 45 as a result, we were unable to find some predictors like viral load, alcohol consumption,
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13 46 smoking, and nutritional history.
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15 47 • The study is not based only on patient cards that started the second-line antiretroviral
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17 48 therapy at the same point in time and also the length of follow up period for each
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19 49 participant was not equal across all participants
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22 50 • The most important strength of this study was the use of longitudinal data analysis. This
23
24 51 ensures a valid estimate by handling data that is measured in different time periods and
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26 52 has missing values.
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29 53 • Besides we had a total of 5380 number of BMI measurements taken from 1016
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31 54 participants with a median BMI of 20.52 Kg/m² (IQR: 18.2; 23). Baseline BMI was
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33 55 calculated for all 1016 patients. These will again strength the precision of the estimates.
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64 **Background**

65 Human Immunodeficiency Virus (HIV) has continued to be a major global public health
66 problem, having killed more than 35 million lives so far. In 2017, 940 000 people died from
67 HIV-related causes globally(1). According to the Ethiopia demographic and health survey of
68 2016, the adult prevalence of HIV in Ethiopia was 0.9%(2). Death due to HIV/AIDS has
69 enormously decreased due to the introduction of antiretroviral therapy (ART) and millions of
70 people are currently accessing it (3).

71 Body weight of HIV patients has been an important diagnostic and evaluation measure. Low
72 BMI is recognized as one of the first criteria for the clinical definition of Acquired Immune
73 Deficiency Syndrome (AIDS). The current World Health Organization (WHO) clinical staging
74 of the disease also includes moderate unexplained weight loss (<10% presumed or measured
75 body weight, stage 2), unexplained severe weight loss (>10% of body weight or BMI \leq 18.5
76 kg/m², clinical stage 3) and HIV wasting syndrome (Unexplained severe wasting, clinical stage
77 4) as criteria to define advanced HIV infection(4).

78 The BMI of HIV patients is an important predictor of ART outcome (5-8), including the
79 prediction of the CD4 cells change (9) and death (10-12). A negative change in BMI of patients
80 was also found to be an independent predictor of dropout from HIV care (13).

81 The BMI of HIV patients is affected by gender(14) duration of treatment (10, 15, 16), Isoniazid
82 Prophylaxis Therapy (IPT) and Cotrimoxazole prophylaxis(CPT) (17, 18) WHO stage (14) and
83 CD4 count (15, 19).

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3 84 This study focused on patients mainly because there is no much option for physicians to choose
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5 85 from if therapy failed. BMI evolution is a very important and easily calculable tool that can
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8 86 predict treatment outcome, dropout from treatment, CD4 recovery, and death, but literatures
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10 87 about its evolution across treatment time and factors affecting it are limited. Therefore this study
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12 88 tried to fill this information gap by determining the BMI evolution over treatment time and
13
14 89 identifying the key factors affecting it. The information generated by this study will contribute to
15
16
17 90 monitoring the response of patients for antiretroviral therapy.

19 91 **Method and materials**

22 92 **Study design and period**

24 93 An institution based retrospective follow up study was conducted among adults, age 24 and
25
26 94 above who are started on second-line ART from February 2008 to February 2016

29 95 **Study area and population**

31 96 The study was conducted in Amhara regional state which is one of the nine administrative
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33 97 regions and two city councils of Ethiopia. The region constitutes majority of ART users in the
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36 98 country. The study population was adult HIV infected patients on second-line ART in all the
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38 99 nine referral hospitals of the region. The first-line treatment consists of a combination of two
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41 100 nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) with one non-nucleoside reverse
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43 101 transcriptase inhibitor (NNRTI). Should failure of first-line treatment occur, a second-line
44
45 102 treatment is implemented, using two NRTIs not previously used in first-line treatment, as well as
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47 103 one additional protease inhibitor (PI).

50 104 **Sample size and sampling procedure**

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3 105 All HIV patients who initiated second-line therapy (n=1233) between February 2008 and 2016
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5 106 were included. But only 1016 patients who had two or more measurements of weight were
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7 107 included in the study.
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10 11 **Data collection procedures**

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13 109 Data extraction checklist was prepared and the data were collected from HIV patient registration
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15 110 cards. Data about the baseline weight and height of patients and other factors like socio-
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17 111 demographic, clinical, and treatment related factors were also collected from each HIV patient
18
19 112 registration card. Adherence to ART was assessed by pill counts at visits and is recorded as
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21 113 'GOOD' ($\geq 95\%$ adherence) or FAIR (80%–95%), while POOR adherence is less than 80% (20).
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26 114 **The data structure, compilation and analysis strategy**

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29 115 The data was cleaned and entered in to EPI info version 7 and analyzed using STATA version
30
31 116 14.0. Body Mass Index (BMI) of HIV patients was computed by dividing the weight of patients
32
33 117 in kilograms (kg) that was recorded during each follow-up visit, to their baseline height in meter
34
35 118 squared. Exploratory data analysis for the weight of patients including individual profile plot,
36
37 119 mean profile plot, descriptive and summary statistics were done. To determine the factors
38
39 120 associated with BMI of patients bi-variable analysis for each independent variable was assessed
40
41 121 and those found to be significant (p-value <0.25) were selected for the multivariable analysis. The
42
43 122 need for random intercept and random slope was checked by likelihood ratio test. Since we
44
45 123 cannot ignore the repeated measurements that have between individual variation and within
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47 124 individual variation(21), linear mixed effect model with random intercept and random slope was
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51 125 fitted.
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54 126 **Patient and Public Involvement**

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3 127 Since we have used secondary data/chart review, patients or the public were not involved during
4
5 128 identifying the research question or design and conduct of the study.
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8 129 **Results**

10 11 130 **Patient characteristics**

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13 131 We had a total of 1016 adult patients who were taking second-line ART. Among the total 538
14
15 132 (52.95%) were males. All participants were above or equal to the age of 24 with a median age of
16
17 133 33 years (IQR: 28; 39). A large proportion of these patients 370 (37.1%) were unemployed and
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19 134 371 (36.9%) have attended secondary school.
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22
23 135 The median follow up time was 18 months (IQR: 5.2; 32.2). There were a maximum of 12 and a
24
25 136 minimum of 2 measurements of weight per patient. Almost all visits were not balanced in their
26
27 137 time of measurements. The median time between each weight measurement is reported as
28
29 138 median (inter-quartile range) as follows: 6.7(4.27, 10.03), 6.08(4.14, 9), 6.13(4.2, 8.9),
30
31 139 6.27(4.53, 8.9), 5.9(3.97, 7.79), 5.83(3.7, 8.13), 5.45 (3.37, 7.43), 4.86(2.97, 6.2), 5.19(3, 7.37),
32
33 140 5.97(4.23, 7.04), 5.55(2.83, 5.86) and 3(0.9, 4.84) months from first to twelfth weight
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35 141 measurement respectively.
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38
39 142 At the initiation of second-line ART majority of participants, 393 (45.54%), were at WHO stage
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41 143 I and 324 (37.54%) were at WHO stage III. A large proportion of participants 871 (86%) had
42
43 144 working functional status at baseline, the rest 114 (11.25%) and 28 (2.75%) were ambulatory
44
45 145 and bedridden respectively. The median CD4 count was 253 (IQR: 147; 399). Isoniazid
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47 146 Prophylaxis Therapy and CPT were given for 247 (24.87%) and 256 (25.65%) of participants
48
49 147 respectively (**Table 1**).
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150 Table-1: Baseline socio-demographic and baseline characteristics of adult HIV patients on ART
 151 in Amhara region, 2008 to 2016

Variables	Frequency	Percentage
Age		
25-34	547	53.89
35-44	333	32.81
45-54	104	10.25
55-64	26	2.56
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Bedridden	28	2.75
CPT given		
No	742	74.35
Yes	256	25.65
INH given		
No	746	75.13
Yes	247	24.87

152 CPT= Cotrimoxazole Prophylaxis Therapy

153 INH= Isoniazid

154

155 **Exploratory data analysis**

156 At baseline a total of 391(38.5%) had BMI of less than 18.5kg/m² whereas 549(54%), 64(6.3%)
157 and 12(1.2%) had BMI 18.5-24.9 kg/m², 25-29.9 kg/m² and ≥ 30 kg/m² respectively.

158 **Individual Profile**

159 The average BMI of patients at baseline was 19.03Kg/m² (SD=3.6 Kg/m²). The minimum and
160 maximum BMI was 8.33 Kg/m² and 33.59 Kg/m² respectively. Before proceeding to the formal
161 statistical analysis we have described the data by exploring how individuals change in BMI over
162 time. To make the individual profile plot more informative and understandable, we have
163 prepared it for the first 100 individuals (**Figure 1**).

164 As we can see from this plot, the BMI status of patients has high variability within an individual
165 over time and among individuals at baseline and also through time. Therefore to fit the data
166 which has variability in the intercept and slope of trajectories very well, we have used a mixed
167 model.

168 **Exploring the mean profile**

169 As stated above all measurements in the data have no similar time of measurements, therefore,
170 we have used smoothing to determine BMI evolution over time (**Figure-2**).

171 As one can easily understand from the mean profile plot the BMI of these patients showed a
172 linear increment over the treatment time.

173 **Modeling the BMI**

174 **Correlation structure checking and model comparison**

175 Comparing with AIC, the correlation structure with the lowest AIC was chosen, in this case the
176 unstructured correlation structure (**Table-2**).

177

178 Table 2: Correlation structure checking and model comparison

	Unstructured	Identity	Exchangeable
AIC	14337.93	17775.34	17666.17
BIC	14531.08	17956.02	17853.09

179
180 The need for random slope and intercept was checked by the likelihood ratio test of model
181 without a random intercept, with only random intercept and a model with both random intercept
182 and slope (**Table-3**).

183 Table 3: Random Effects Models comparison

Random effects	LR X ²	P value
Model-1 intercept	5625.28	0.0000
Model2 intercept, time	297.13	0.0000

184 So as we can see from the table-3 the inclusion of random intercept and random slope is
185 reasonable so in the final model, we have used both random intercept and random slope.

186 **Factors affecting BMI evolution over time**

187 Based on the multi-variable output: time since the start of second-line ART, IPT, CPT,
188 Functional status, WHO stage of the disease; and time interaction of categorical variables like
189 educational status, functional status and WHO stages were found to be significant predictors of
190 the BMI evolution of HIV patients on second-line ART.

191 Keeping all the other variables constant, for a one month increment in the treatment duration the
192 BMI of a patient increases by 0.013 kg/m². But specifically individuals who have tertiary level of
193 education, ambulatory functional status and who are at WHO stage III have additional 0.02
194 kg/m², 0.03 kg/m² and 0.01 kg/m² increment respectively in their BMI for a one month
195 increment in the duration of treatment.

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3 196 Taking IPT and CPT increases BMI by 0.87 kg/m² and 0.63 kg/m² respectively as compared to
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5 197 their counterparts. Nongovernmental organization employment was associated with a 2.02 kg/m²
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7 198 increment when compared to government employment. Patients who are ambulatory and
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9 199 bedridden in their functional status have a 1.16 kg/m² and 1.83 kg/m² decrement in their BMI as
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11 200 compared to those who have working functional status. Patients who are at WHO stage III and
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13 201 WHO stage IV have decreased BMI by 0.42 kg/m² and 0.62 kg/m² when compared with those
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15 202 who are at WHO stage I. For a unit cell/mm³ increase in the CD4 count of patients BMI was
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17 203 found to increase by a factor of 0.001 kg/m² (**Table-4**).
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204 **Table 4: Parameter estimates for full linear mixed effect model**

Characteristics	Co-efficient	Confidence interval		P value
		Lower	Upper	
Intercept	19.03	18.11	19.95	0.000
Sex				
Female	0.00			
Male	-0.01	-0.51	0.48	0.956
Adherence				
Poor	0.00			
Fair	0.43	-0.24	1.10	0.207
Good	0.48	-0.06	1.02	0.080
INH prophylaxis given				
No	0.00			
Yes	0.87	0.32	1.42	0.002
CPT prophylaxis given				
No	0.00			
Yes	0.63	0.08	1.19	0.025
Educational status				
Illiterate	0.00			
Primary	-0.03	-0.72	0.66	0.924
Secondary	0.47	-0.21	1.14	0.176
Tertiary	0.94	-0.01	1.89	0.051
Functional status				
Working	0.00			
Ambulatory	-1.16	-1.95	-1.31	0.000
Bedridden	-1.83	-2.47	-1.21	0.000
WHO stage				
Stage I	0.00			
Stage II	-0.06	-0.45	0.33	0.760
Stage III	-0.42	-0.65	-0.20	0.000
Stage IV	-0.62	-1.02	-0.22	0.002
CD4 count	0.001	0.0008	0.0015	0.000
Time on treatment	0.013	0.004	0.022	0.005
Education status x time				
Illiterate x time	0.00			
Primary x time	0.002	-0.01	0.015	0.801
Secondary x time	0.01	-0.003	0.019	0.162
Tertiary x time	0.02	0.01	0.04	0.009
Functional status x time				
Working x time	0.00			
Ambulatory x time	0.03	0.01	0.05	0.000
Bedridden x time	-0.01	-0.05	0.02	0.49
WHO stage x time				
Stage I x time	0.00			
Stage II x time	0.004	-0.008	0.166	0.522
Stage III x time	0.01	0.007	0.020	0.000
Stage IV x time	-0.0003	-0.016	0.016	0.969

205 **DISCUSSION**

206 This study has found a linear increment in BMI of patients over the treatment time. There were
207 BMI differences between subjects at baseline and in their progress over time.

208 Factors found to have a significant effect on the evolution of BMI over treatment time at a
209 multivariable linear mixed effect model were, second-line treatment duration, IPT, CPT,
210 employment status, functional status of the patient, WHO stage, and CD4 count. Time
211 interaction of educational status, functional status, and WHO stage were also significant
212 predictors of BMI evolution.

213 For a one month increase in treatment duration the BMI of patients increases by 0.04 kg/m², this
214 finding is in line with studies in South Africa (22), India (10) and United States(23). The possible
215 reasons for weight gain could be due to normal reversion of the weight loss associated with HIV
216 or due to drug related metabolic changes which include hyperlipidemia, insulin resistance and
217 diabetes(24).

218 Patients who took IPT and CPT have increased BMI when compared to their respective
219 counterparts. This finding is supported by other studies like a study conducted in Abidjan (17)
220 and multicenter controlled clinical trial in Africa (18). The main reason for this association could
221 be the reduction of potentially disabling and wasting diseases like tuberculosis and other
222 opportunistic infections by these prophylactic drugs(25, 26). A highly increased appetite by the
223 prophylaxis could also be possible explanation(27).

224 Being ambulatory and bedridden decreases BMI when compared to those who are working.
225 Since these individuals are not working they may not access a nutritious and balanced diet which
226 affects their BMI. Another reason could be additionally decreased immunity caused by physical
227 inactivity which makes them more susceptible to minor infections leading to a higher calorie

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3 228 loss(28). These groups of people are also at higher risk of diarrheal disease for they cannot take
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5 229 care of themselves which in turn causes weight loss(29).
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8 230 When compared with patients who are at WHO stage I, those who are at WHO stage III and
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10 231 WHO stage IV have decreased BMI. This finding is in line with a multicenter study in resource
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12 232 limited settings which shows individuals who had high clinical status (WHO stage III and WHO
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14 233 stage IV) had poorer weight gain when compared to weight change in patients at lower WHO
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16 234 stage(14). The possible reason can be unexplained chronic diarrhea and HIV enteropathy in these
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18 235 patients and the associated malabsorption (4) or it could be due to pyrexia of unknown origin in
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20 236 the late stage of the disease which results an increased calorie loss and wasting(30).
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24 237 The BMI of patients was also found to increase with the increment in their CD4 count. This
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26 238 positive association is supported by evidence from a study conducted in Boston (15) and
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28 239 Tanzania (19). This increment can be explained by the association of increment of CD4 count
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30 240 with good clinical changes like viral suppression, improved immunity, and appetite leading to
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32 241 increment in the BMI of patients (13).
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35 242 Being a retrospective study, this study shares the limitations of secondary data collection, as a
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37 243 result, we were unable to find some predictors like viral load, alcohol consumption, smoking,
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39 244 marital status, and nutritional history. The data may also have errors in documenting weight
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41 245 among some measurements.
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44 45 246 **CONCLUSION**

46
47 247 In this study, we have found a linear increment in the BMI of HIV patients on second line ART.
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49 248 There was a significant variation of BMI of patients at baseline and through ART treatment time.
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51 249 Duration of treatment, IPT, CPT, functional status, WHO stage of the disease, CD4 count; and
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53 250 time interaction of categorical variables like educational status, functional status and WHO
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3 251 stages were found to be significant predictors. The positive change in the BMI of patients shows
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5 252 an encouraging trend for we know this has a positive impact on the CD4 recovery, decrease lost
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8 253 to follow up and death. Clinicians also must consider the identified risk factors when they
9
10 254 provide service for these patients.

11 255 **ABBREVIATIONS**

12
13
14 256 AIC, Akaike's Information Criterion; AIDS, Acquired Immunodeficiency Virus; ART,
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16 257 Anti-Retroviral Therapy; BIC, Bayesian information criterion; BMI,
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19 258 Body Mass Index; CI, Confidence Interval; CPT, Co-trimoxazole preventive therapy; HIV,
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21 259 Human Immuno Deficiency Virus; IPT, Isoniazid Prophylaxis Therapy; Kg,
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24 260 Kilogram; NGO, Non Governmental Organizations; WHO,
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26 261 World Health Organization.

27 28 29 30 **Declarations**

31 32 **Ethical consideration**

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35 264 Ethical clearance was obtained from the Institutional Review Board of Institute of Public Health,
36
37 265 University of Gondar. Names and unique ART numbers of patients were not collected to keep
38
39 266 the privacy of patients during the data collection.

40 41 **Availability of data and materials**

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44 268 The data upon which the result based could be accessed by a reasonable request made to the
45
46 269 corresponding author.

47 48 **Funding**

49
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51 271 Not applicable

52 53 **Consent for publication**

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3 273 Not applicable
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6 274 **Competing interest**
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8 275 Authors declare that they have no conflict of interest
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10
11 276 **Authors' contribution**

12
13 277 AGB, LDG, EGZ, TAA, ATT have actively participated during Conception and design,
14
15 278 acquisition of data, analysis, and interpretation of data. All authors have read and approved the
16
17 279 final version of the manuscript.
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19
20 280 **Acknowledgments**

21
22 281 First of all, we would like to thank the Almighty God. We would like say thank you to
23
24 282 University of Gondar, Amhara regional health office and hospital administrative bodies,
25
26 283 clinicians, data clerks, and card room workers for their cooperation and permission to conduct
27
28 284 the study.
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32 285 **Authors' information**
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34 286 Adhanom Gebreegziabher has BSc degree in Public health as a background and second degree in
35
36 287 Epidemiology and Biostatistics; now teaching Epidemiology, Research methodology and
37
38 288 communicable disease control courses in the University of Gondar, Ethiopia
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4 382 **Figures Legend**

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7 383 **Figure 1: Individual profile plot of BMI overtime for the first 100 individuals at second-**
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9 384 **line ART in Amhara region, 2008-2016**

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12 385 **Figure 2: Time plot of BMI versus treatment time in months with lowess smoothed curve**
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14 386 **superimposed for HIV patients on second-line ART in Amhara region 2008-2016**

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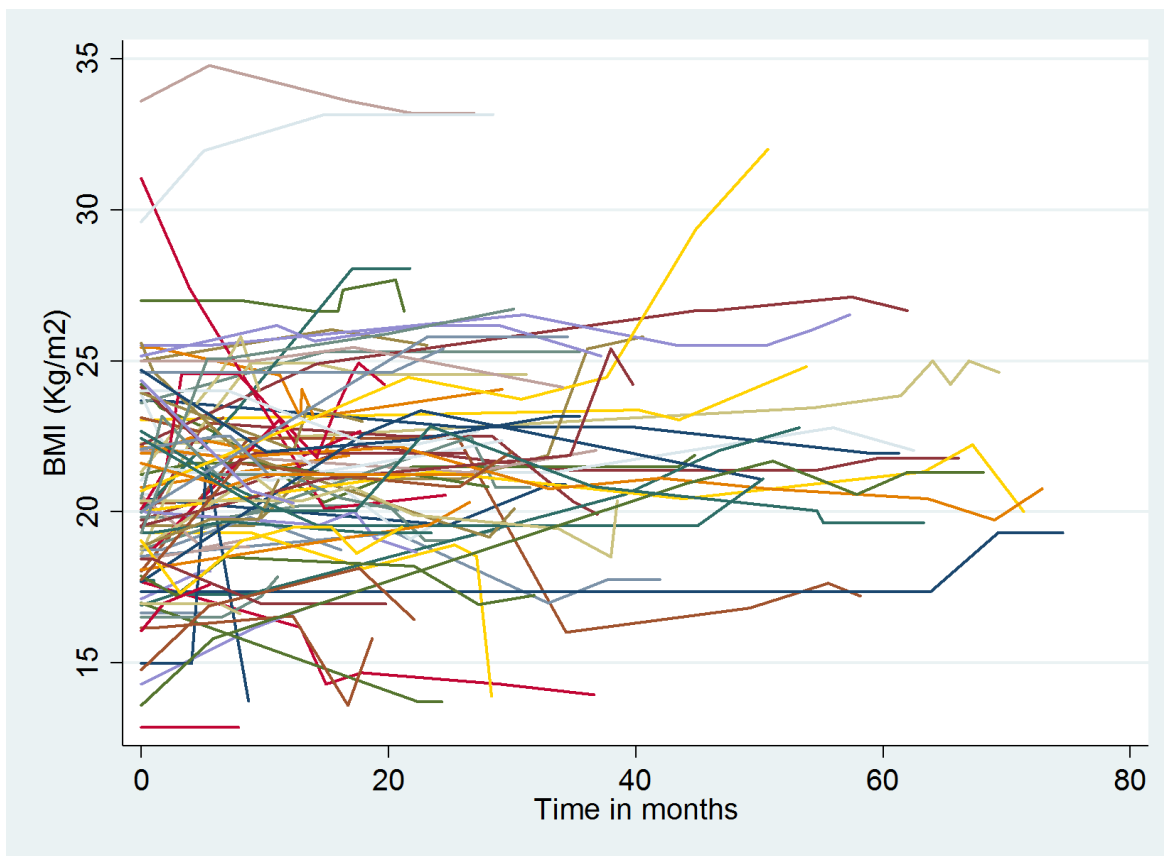
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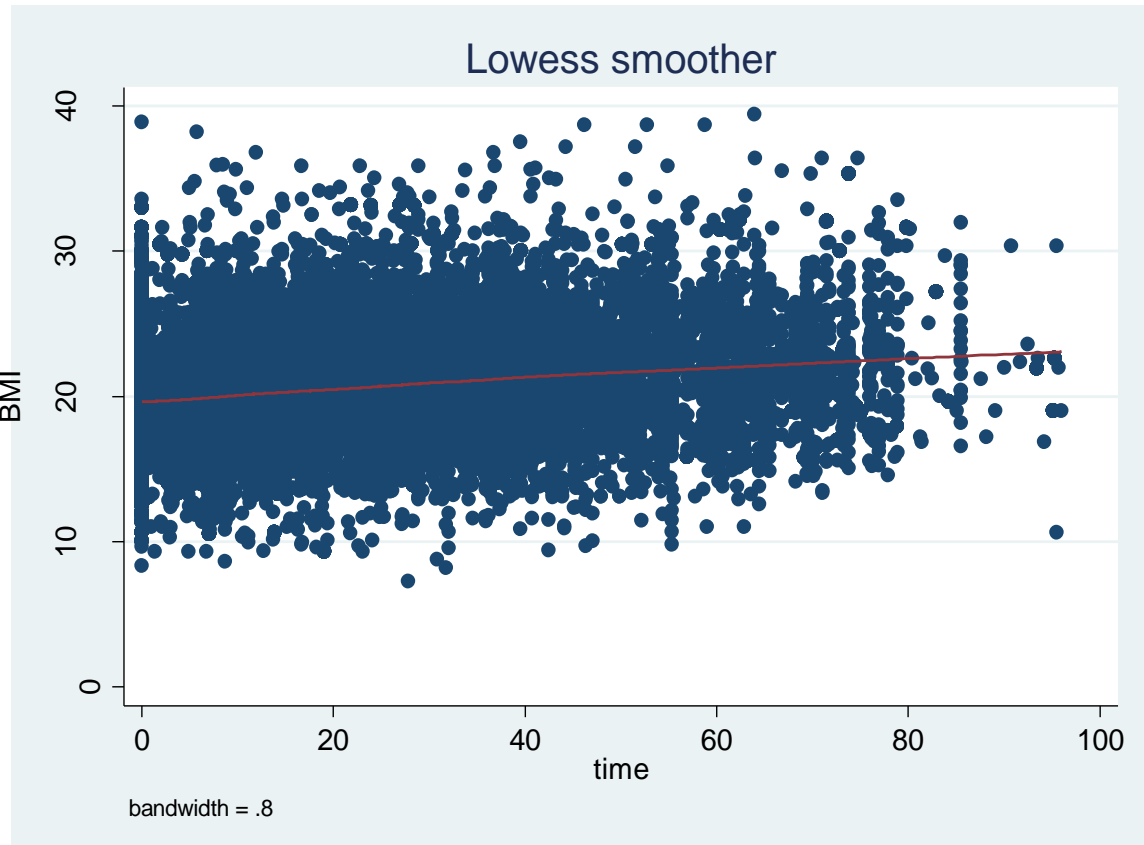
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STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (b) For matched studies, give matching criteria and number of exposed and unexposed	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) If applicable, explain how loss to follow-up was addressed (e) Describe any sensitivity analyses	6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	Report numbers of outcome events or summary measures over time	7-9

1	Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-9
2			(b) Report category boundaries when continuous variables were categorized	
3			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
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9	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	--
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11	Discussion			
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13	Key results	18	Summarise key results with reference to study objectives	9-10
14	Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
15				
16	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10 - 11
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18				
19	Generalisability	21	Discuss the generalisability (external validity) of the study results	11
20				
21	Other information			
22	Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12
23				
24				

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.