

**USE OF ACTIVE FORM OF HYDROGEN CONTAINED STEAM H(H₂O) AS THERAPY
AND ITS OUTCOME IN HEMODIALYSIS PATIENTS****Dr. Kishore Kumar***

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ABSTRACT

Chronic kidney disease is one of the major contributors of increased burden of non-communicable diseases worldwide with prevalence of 9.1% to 23%. Increased life expectancy and treatment development of Diabetes and Hypertension are leading contributors with increasing tendency to development of ESRD population need RRT (Renal replacement therapy) in the form of dialysis and transplant. Hemodialysis contributes to major part of RRT. Hemodialysis and CKD with ESRD and its causes are pro-inflammatory conditions, which increase number of oxidative radicals and they activate a cascade of inflammation in human body. Inflammation ideally a protective mechanism of human body against any foreign invasion gets worse due to accumulation of oxidative radicals e.g., O³, -OH³, IL6, IL1, TNF-β, FGF-β etc. Once this inflammatory cascade starts it get uncontrolled with time and results with some severe structural, hormonal, biochemical and hemostatic complication with resultant worse nightmare of many nephrologists due to uncontrolled B.P, blood sugar and Hemoglobin. Patients' quality of life decreases, and one becomes dependent. To resolve this issue a prospective study was done by using mixture of active form of hydrogen contained steam (H(H₂O)m) with nasal cannulas for three (3) hours session for three (3) days per week during hemodialysis at Renasense dialysis unit in Seychelles. Total 23 patients fulfilled inclusion criteria, 21 were on hemodialysis and two others, were studied. Major changes we focused were control of hypertension, diabetes, hemoglobin, and impact on quality of life. Out of 20 hypertensive patients initially 14 were using multiple drugs and 6 single drugs, after 6 months of continues therapy both diastolic and systolic blood pressures were well controlled and medicine quantity was minimized. Out of 6 diabetic patients, 4 were using OHGs and 2 were on insulin, after 6 months of treatment HBA1C stabilized at 6.5 to 7 and OHGs and insulin doses were reduced to stopped. Similarly, improvement in hemoglobin levels were also noticed and it was stabilized at 11 to 13mh/dl, at higher levels of Hb external erythropoietin administration was also reduced. Overall, with the continues treatment of H(H₂O), quality of life in all participants became better and few of them were resumed their work and actively participated in community development. In this study we concluded that consecutive use of H(H₂O)m for 3 months and more efficiently reduces oxidative stress and controls the inflammatory cascade with resultant improvement in quality of life by controlling metabolic, functional, and hormonal body parameters. Despite all satisfactory results, small number of participants and lack of prior awareness and reluctance to accept new therapy, were major limitations. So, we recommended to do a large population-based study and explore more potential effect of H(H₂O)m to reduce increased oxidative stress in today's world.

KEYWORDS: Chronic Kidney Disease, End Stage Renal Disease, Hemodialysis, HydrogenTherapy.**INTRODUCTION**

Chronic kidney disease is one of the leading non-communicable chronic disease ranked 5th to 9th in different regions and to one estimation its global incidence and prevalence is continuously increasing in all regions ranging from 9.1% to 23%.^[3] A major increase in CKD prevalence was noted in recent years with increase in life expectancy due to advancement in treatment of diabetes and hypertension, which are leading non-communicable chronic disease and major causes of CKD.^[1,2,4,5] Increase in CKD increased the population of

End stage renal diseases with subsequent available candidates for Renal replacement therapy in the form of dialysis and renal transplant, Only available to 3% to 17% in low income countries and 41% to 60% in high income countries.^[5,6] Of these renal replacement therapies dialysis and of which hemodialysis is main modality of treatment.^[7] CKD, Diabetes, ESRD and hemodialysis all being pro-inflammatory conditions starts vicious inflammatory cascades and increase oxidative stress which alters human body's normal response to these inflammatory reactions.^[9,10,20] This

results in biochemical, structural, hormonal and nutritional changes in body which leads to complications include, anemia, fluctuation in blood pressure, bone diseases, mental as well as physical fatigue and difficult to manage diabetes in Dialysis and ESRD patients.^[11] Due to these complications' patients experience early aging and deterioration in active lifestyle which further leads to deterioration in this already frail population leads decrease in active society contributors.^[13] Different novel therapies are in continuous research to decrease these inflammatory milieu and reduce complications, but no one is seeing final approach.^[8]

Different studies have been done to determine biomarkers which play key role in inflammation and their association to CKD. Markers which are considered as basics of any inflammatory process in body are raised levels of CRP, IL-6, TNF- α , TGF- β and FGF- β etc. and oxidative radicals like O³, OH³ and OH are major contributors for chronic inflammation associated morbidity and mortality.^[14,21-26] Two different studies conducted by Eustace JA et. all in 2004 and Stenvinkel P et. all in 2002 described that raise in CRP and a study by Sun J, Axelsson J et. all shown that raised in IL-6 was directly associated with progression of CKD and their levels raise even more in patients on dialysis which leads to all chronic complication.^[15,16,17] All efforts are being done and still in research are directed towards decrease reduction these inflammatory markers and oxidative stress to improve the quality of life these patients.

There are many antioxidants and immune regulators in use, but it was shown that hydrogen have magnificent antioxidant, anti-inflammatory immunomodulation effects and neutralizing effects which are more beneficial than its explosive use.^[2] Hydrogen which is the lightest, smallest colorless, odorless, tasteless, and non-toxic particle on earth and hardly found alone because of its very unstable nature so it always comes in compounds and major source of which is water.^[28] Hydrogen is an explosive gas when its concentration in air reaches 4% to 75% but when it is less than 4% it does not possess explosive qualities which make it more suitable for living things.^[29,30] The Effectiveness of hydrogen in medical field have been studied since 1970 and this process is still going on to know its role as treatment and reduction of complications of different medical problems.^[19] There are different methods of using hydrogen as medical treatment including inhalation of hydrogen gas, ingestion hydrogen-rich water (HRW), and inoculation of hydrogen-rich saline (HRS) with different effectiveness.^[31,32,33] But use of hydrogen gas in <4% quantity have shown impressive results in reducing oxidative stress and inflammation with improvement in patient's morbidity and mortality parameters.^[34]

After reviewing so many articles on hydrogen therapy and a study by Nakayama M, et.all showed that Administration of H₂ to hemodialysis (HD) solutions improves uremia and associated uncontrolled

inflammatory reactions with control blood pressure (BP) in Hemodialysis population.^[35] This provoked a unique treatment opportunity and its benefits in hemodialysis and CKD associated complications. To testy this we thought to use hydrogen Gas with an object to observe improvement in HB levels, control of BP and HBA1C in diabetes, among CKD, hemodialysis other chronic conditions along with overall emphasis on quality of life. Among different methods of hydrogen therapy, we used Hydrogen Therapy as Mixture of Active Form of Hydrogen Contained Steam (H(H₂O)m) generated by equipment Suisonia (A machine developed by Suisonia Co., Ltd (Suisonia, FRJ-003, Kitakyushu, Japan. Previously designated as XEN). Suisonia was used in this study, because of its safety in hydrogen concentration of 0.1% to 0.3%, while being convenient in use via nasal cannula, with an object to see its effect on HD related complications.

MATERIALS AND METHODS

Study Design: Prospective Study

Study Populations: All registered patients who are already undergoing Hemodialysis and other patients with chronic conditions

Study Duration: One year from the start of study JUNE-2018 TO JUNE-2019

Treatment Modality Used: Hydrogen Therapy as Mixture of Active Form of Hydrogen Contained Steam Gas (H(H₂O)m) produced by SUISONIA, it's a machine developed by Suisonia Co., Ltd (Suisonia, FRJ-003, Kitakyushu, Japan)

Inclusion Criteria: All those patients who give consent for volunteer participation in study

Exclusion Criteria: All those who are not willing to participate in study

Study Setup: Advanced Renal Care (Renasense) Seychelles Unit

Data Collection Procedure

After getting informed consent from registered patients at Renasense Dialysis unit in Seychelles for Hemodialysis and CKD treatment regardless of cause and time duration, and others with chronic conditions who volunteered and agreed on terms who fulfill inclusion criteria were included in study. Base line tests done before commencement of study were BLOOD CP, HBA1C, and BLOOD PRESSURE, the same were recorded on monthly basis and trend of change in values of hemoglobin, sugar levels and blood pressure were studied.

Hydrogen Administration and Generation Method

An active form of hydrogen contained steam gas (H(H₂O)m) generated by Suisonia by using sterile water

steam and metal (Fe) heated to over 600°C within a tube chamber.

1) Mechanism of Active Hydrogen Generation

First, suisonia heater generates steam bypasses through the heated chamber, the heated metal (Fe) will capture oxygen (O) from contacted steam (H₂O) oxidizing Fe and leaving hydrogen (H) in the chamber. At the same time, there are H₂O molecule steam in the same chamber space that did not contact with heated Fe will surround the hydrogen resulting to H(H₂O)m.

2) Amount of Hydrogen Transported

Additionally, Suisonia pulls air from outside to assist inhalation, making its concentration of hydrogen inside the device approximately 2.4% as confirmed by the portable hydrogen detector. After the generated H(H₂O)m travels through nasal cannula which is specifically designed and provided with equipment was used for administration of hydrogen at provided concentration of 0.1% to 0.3% when inhaled for total dose of three (3) hours session for three (3) days per week during the same time of Hemodialysis session for patient's comfort and time saving.

Data Analysis

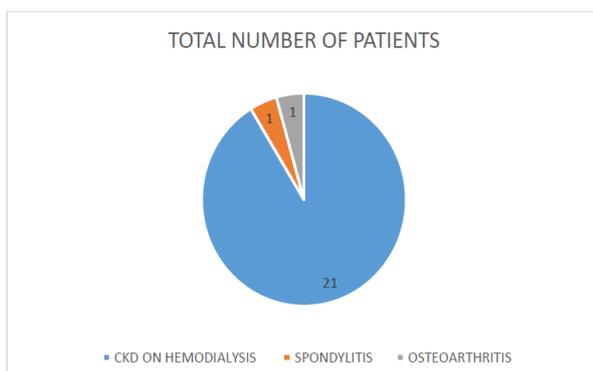
SPSS software was used to analyze the data and results were concluded as changes in values of base line parameters which were projected in graphs and diagrams. Numerical variables like HB levels, HBA1C levels and BLOOD PRESSURE reading were compared at start and end of study and chi-square test was used to see the significance. P value of <0.05 was considered insignificant

OBJECTIVE OF STUDY

The effect of H(H₂O)m generated by SUISONIA on HB, BP and HBA1C on hemodialysis, CKD, and other chronic disease patients.

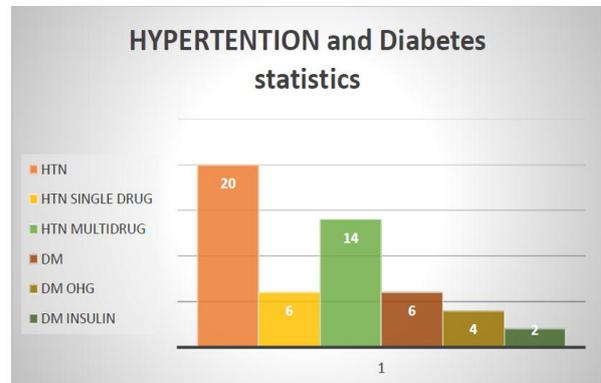
RESULTS

In our study total number of patients included were 23, out of which 21 patients having CKD5 undergoing hemodialysis and two were having other chronic problems Osteoarthritis and spondylitis as shown in figure 01



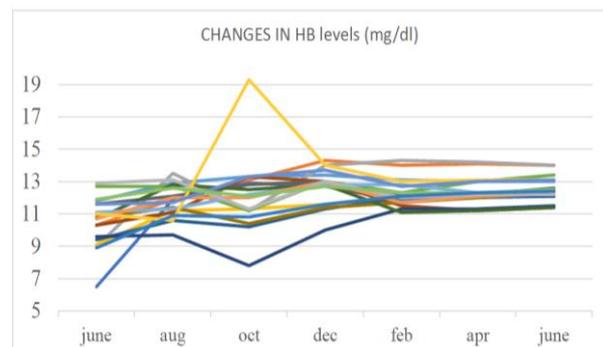
Among 23 patients 20 patients had Hypertension and 6 had diabetes as comorbid conditions. Among hypertensive patients 06 were using single drug to control their BP and 14 were using multidrug regime.

Out of 6 diabetes patients 02 were on oral hypoglycemic drugs and 04 were using insulin. As shown is graph below

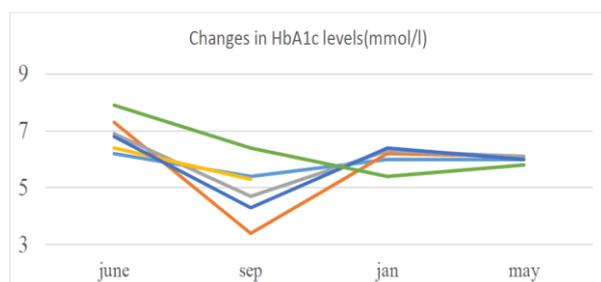


Main parameters which were studied during study were changes in Hemoglobin levels, Hypertension and Diabetes control by measuring HBA1C levels and improvement in quality of life in all patients.

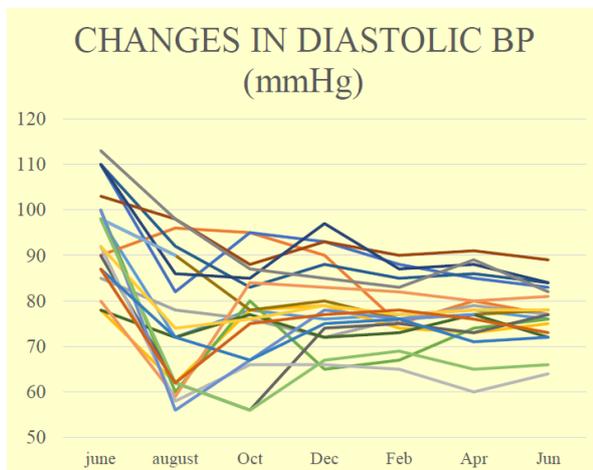
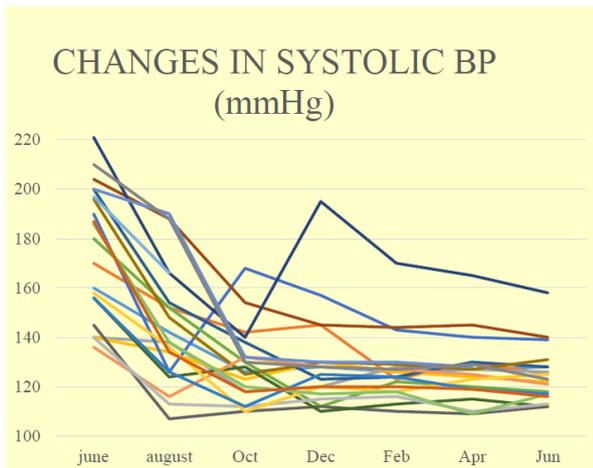
After using steam generated hydrogen gas therapy there was significant improvement in hemoglobin levels with time and stabilized at 11 to 13 mg/dl over 6 months of continues treatment. The p value was <0.05 which was insignificant as shown in graph below



Similarly, after initial fluctuations in levels of HBA1C it stabilized after 6 months at levels of 6.5 to 7 and p value was <0.05 which was not significant as shown in graph below



Hypertension was also controlled and many of patients shown decrease in both systolic and diastolic blood pressure which was stabilized after 6 months in most of patients as shown in graph below



Along with these results we were also noted that all patient's quality of life improved and postdialysis exhaustion symptoms were also resolved.

DISCUSSION

Global burden of chronic diseases are main concerns for now days for increased burden on healthcare both financially and physically because of increase in aging population, unbalanced change lifestyle, global environmental deterioration.^[37] Among chronic diseases diabetes mellitus, hypertension, COPD, cancer is one of the prominent causes of global morbidity and mortality.^[36] To one estimation currently 71% of mortality is due to chronic conditions with 46% increase since in 2001 and it is even more in developing countries i.e. 70% of population become socially inactive or die before the age of 70 years and even lower.^[37] to one estimation by 2030 this rate will increase further and it will not be unwise to say that chronic disease is a silent global pandemic.^[37] with continuous increase in number of diabetes and hypertension increasing there is

proportionally improvement in their treatment which increased life expectancy and expected complications on of major complication is development of CKD.^[1,2,4,5] Increase in CKD population there is proportionally increase in the population of End stage renal diseases with subsequent available candidates for Renal replacement therapy.^[38] one of the main modality of RRT is dialysis and its major contribution is in the form of hemodialysis, which itself is another source of chronic disease related mortality and morbidity.^[39] CKD and H.D along with diabetes and hypertension are associated with generation of abnormal oxidative environment in body due to which normal inflammatory process becomes uncontrolled and lead to severe complications which enhance the mortality and morbidity ratios.^[20]

Until now various inflammatory markers and oxidative radicals studied which are considered as culprits of uncontrolled vicious cycle of complications in living beings. Out of them major contributors are increased levels of inflammatory markers are IL-6, CRP, D. DIMERS, TNF- α , TNF- γ , TGF- β and oxidative radicals such as O⁺, OH⁻, OH⁺³ etc.^[14-17,21-26] Any step to reduce these markers inflammatory and oxidative markers leads to improvement in patients' health and quality of life.^[40] Numerous antioxidants are already studies and still understudy for reducing oxidative stress and inflammation, among them hydrogen gas is also considered one of the potential antioxidant molecules.

Hydrogen gas described as potent antioxidant since its effect was observed first in 1970 and 1975 by using hydrogen rich alkaline water and helium.^[43,44] Since then it is continuously researched in animal and human studies to explore its potentials. Hydrogen gas is maker of 75% of universe mass and little of its amount present in earth's atmosphere. It is very unstable and always found as water of inorganic or organic compound. The biologically safely effective level of hydrogen was considered < 4% of concentration of hydrogen gas in any compound.^[18]

In all studies since 1970 till now worldwide it is universally observed that Hydrogen, being tiny particle in universe and easy dispersible quality across all types of cell membranes, serve as strong antioxidant molecule and its importance in medical field is not widely honored as other commercially available antioxidant. Japan which is considered as pioneer of hydrogen therapy since 1970s have developed many equipment and methods of hydrogen delivery and still this exposure is going on. Of all methods different concentration of hydrogen is being released for medical treatment and every method showed its potential of reduction in oxidative stress, inflammation, and control of cytokine storm. Among different methods of hydrogen therapy, use of hydrogen gas was considered safest mode of hydrogen delivery and latest equipment developed by SUISONIA have minimum effective concentration of hydrogen gas combined with water vapors termed as Mixture of Active

Form of Hydrogen Contained Steam Gas (H(H₂O)m) formerly known as Xen. We have used this equipment in our study. It generates hydrogen by using sterile water steam and metal (Fe) heated to over 600°C within a tube chamber. By its specific mechanism i.e. (suisonia heater generates steam from water and when it passes through the heated chamber, the heated metal (Fe) will capture oxygen (O) from contacted steam (H₂O) oxidizing Fe to Fe₂O₃ and leaving hydrogen (H) in the chamber. At the same time, leftover H₂O molecule steam in the same chamber space that did not contact with heated Fe will surround the hydrogen resulting to generation of active hydrogen in the form H(H₂O)m. Additionally, Suisonia pulls air from outside to assist inhalation) The concentration of hydrogen present inside the device is approximately 2.4% and when it is finally delivered to human body through nasal cannula its concentration reaches to 0.1% to 0.3% at the end of nasal canula which determines that it is minimum effective quantity of H(H₂O)m.

In a recent study by Shao-Ting Wang, Cheng Bao et.al in China showed that use of hydrogen steam generated by SUISONIA equipment was effective in reducing in reducing lung inflammation markers of IL-6, TNF- α and CRP in asthma and COPD. Another study in Japan by Toru Ishagashi also formulated same results with high insight of mechanism of reducing oxidative and inflammatory stress which supported our hypothesis that by reducing oxidative stress chronic disease related complications can be controlled in CKD on hemodialysis patients.^[41,42] These studies favors our hypothesis that by implication of hydrogen therapy CKD and Dialysis associated oxidative and inflammatory activations are controlled and cause improve in these patient's quality of life.

Treatment with hydrogen gas inhalation and other forms of hydrogen therapy showed markedly improvement in metabolic profile in patients with Diabetes, metabolic syndrome, and obesity. This is concluded by Kajiyama S, Hasegawa G, et. all in 2008 and recently by Ming, Y., Ma, and Q. H in 2020, in their studies and showed significant improvement and control of lipid profile, HBA1C levels and uric acid concentration by possible mechanism of control of oxidative stress.^[45,46] It intensifies our hypothesis and results obtained from this study which shows reduction in HBA1C level after three months continues inhalation of H(H₂O)m and improvement in associated complications of fluctuation in HBA1c and glucose results.

Inhalation of H(H₂O)m not only improved metabolic profile of our patients but also helped in control of B.P, which is a nightmare of any nephrologist. By continues inhalation of H(H₂O)m for 3 months there was dramatic improvement in Blood pressure of all patients, and this also compelled us to reduce their antihypertensive medicines. This effect is widely seen in studies conducted on both animal and human models. Studies

which support our study hypothesis and have almost same results were conducted by Sugoi et.al in 2013, Suno. m et.al in 2018 and Dixon et.al in 2020.^[47,48, and 49]

The effect of hydrogen was also seen in improvement of hematological conditions such as anemia, blood cancers and hormonal deficiency this is confirmed by significant number of studies such as a study by Nakayama, Masaaki et al. showed that with supplementation hydrogen gas during hemodialysis not only reduces frequency of dialysis related complications but also improved their HB level, Blood pressure and uremic pruritus.^[50,51] This support our study results that after consecutive inhalation of hydrogen as supplement during each hemodialysis improved their HB level and reduces the requirement of supplemental erythropoietin.

After continues inhalation of hydrogen for three consecutive days many patients communicated that their life has dramatically changes, they felt more energetic and love to resume their work which was left due to dialysis and CKD related complications. There was reduction in their complain of easy fatigability, restlessness, irritation, and sense of being burden on family.

Overall, every patient has been benefitted in terms of improvement in their quality of life.

CONCLUSION

With this study we concluded that supplementation hydrogen gas (mixture of hydrogen contained steam gas) during every dialysis have improved patient's outcome of biochemical, hormonal, and physical parameters.

LIMITATION

It was a small prospective study, and many patients were initially shown reluctance to include in study.

RECOMMENDATION

After reviewing so many articles on potential benefits of Hydrogen therapy we recommend more studies in chronic disease population on large scale and promote its use as safest antioxidant in future.

REFERENCES

1. Global, Regional, And National Burden and Trend of Diabetes In 195 Countries and Territories: An Analysis From 1990 To 2025 Xiling Lin^{1, 4}, Yufeng Xu^{2,4}, Xiaowen Pan^{1,4}, Jingya Xu¹, Yue Ding¹, Xue Sun³, Xiaoxiao Song¹, Yuezhong Ren¹ & Peng-Fei Shan^{1*}
2. IDF Diabetes Atlas Ninth Edition 2019.
3. The Global Burden of Chronic Kidney Disease, *Paul Cockwell, Lori-Ann Fisher, The Lancet, February 29, 2020; 395.
4. Webster AC, Nagler EV, Morton RL, Masson P Chronic Kidney Disease. Lancet, 2017; 389: 1238-

- 1252.
5. United States Renal Data System. 2018 USRDS Annual Data Report: Epidemiology of Kidney Disease in The United States. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2018.
 6. "The Epidemiology of Renal Replacement Therapy in Two Different Parts of The World: The Latin American Dialysis and Transplant Registry Versus the European Renal Association-European Dialysis and Transplant Association Registry." Luxardo, Rosario Et Al. *Revista Panamericana De Salud Publica = Pan American Journal of Public Health*, 7 Sep. 2018; 42 E87. Doi:10.26633/RPSP.2018.87
 7. International Society of Nephrology. *Global Kidney Health Atlas*. 2nd Ed. 2019. Available From: <https://www.theisn.org/initiatives/global-kidney-health-atlas>. Accessed 2020 Nov 17.
 8. Chronic Inflammation In End-Stage Renal Disease And Dialysis Gabriela Cobo,¹ Bengt Lindholm,² And Peter Stenvinkel², *Nephrol Dial Transplant*, Oct, 2018; 33(3): Iii35–Iii40.
 9. Inflammation And Protein-Energy Wasting in The Uremic Milieu. Jankowska M, Cobo G, Lindholm B, Stenvinkel P, *Contrib Nephrol*, 2017; 191: 58-71.
 10. Association Between Albuminuria, Kidney Function, And Inflammatory Biomarker Profile in CKD In CRIC. Gupta J, Mitra N, Kanetsky PA, Devaney J, Wing MR, Reilly M, Shah VO, Balakrishnan VS, Guzman NJ, Girndt M, Periera BG, Feldman HI, Kusek JW, Joffe MM, Raj DS, CRIC Study Investigators. *Clin J Am Soc Nephrol*, Dec., 2012; 7(12): 1938-46.
 11. Bello, A. K., Alrukhaimi, M., Ashuntantang, G. E., Basnet, S., Rotter, R. C., Douthat, W. G., Kazancioglu, R., Köttgen, A., Nangaku, M., Powe, N. R., White, S. L., Wheeler, D. C., & Moe, O. Complications Of Chronic Kidney Disease: Current State, Knowledge Gaps, And Strategy for Action. *Kidney International Supplements*, 2017; 7(2): 122–129.
 12. Egan, Brent M.A; Kjeldsen, Sverre E.B; Grassi, Guidoc, D; Esler, Murraye; Mancina, Guiseppef the Global Burden of Hypertension Exceeds 1.4 billion People, *Journal of Hypertension*, June, 2019; 37(6): 1148-1153.
 13. Kooman JP, Dekker MJ, Usvyat LA. Et Al. Inflammation and Premature Aging in Advanced Chronic Kidney Disease. *Am J Physiol Renal Physiol*, Oct-01, 2017; 313: F938–FF50.
 14. Origin And Physiological Roles of Inflammation. Medzhitov R *Nature*, Jul 24, 2008; 454(7203): 428-35.
 15. Prevalence Of Acidosis and Inflammation and Their Association with Low Serum Albumin in Chronic Kidney Disease. Eustace JA, Astor B, Muntner PM, Ikizler TA, Coresh J *kidney Int*, Mar., 2004; 65(3): 1031-40.
 16. Inflammation And Outcome in End-Stage Renal Failure: Does Female Gender Constitute a Survival Advantage? Stenvinkel P, Wanner C, Metzger T, Heimbürger O, Mallamaci F, Tripepi G, Malatino L, Zoccali C *kidney Int.*, Nov, 2002; 62(5): 1791-8.
 17. Biomarkers Of Cardiovascular Disease and Mortality Risk in Patients with Advanced CKD. Sun J, Axelsson J, Machowska A, Heimbürger O, Bárány P, Lindholm B, Lindström K, Stenvinkel P, Qureshi AR *Clin J Am Soc Nephrol*, Jul 7, 2016; 11(7): 1163-72.
 18. Recent Progress Toward Hydrogen Medicine: Potential of Molecular Hydrogen for Preventive and Therapeutic Applications Shigeo Ohta* Department of Biochemistry and Cell Biology, Institute of Development and Aging Sciences, Graduate School of Medicine, Nippon Medical School *Current Pharmaceutical Design*, 2011; 17: 2241-2252.
 19. Abisso TG, Adzavon YM, Zhao P, Zhang X, Liu M, Ma X, et al. Current Progress in Molecular Hydrogen Medication: Protective and Therapeutic Uses of Hydrogen Against Different Disease Scenarios. *Intern Med.*, 2020; 10: 314. Doi: 10.35248/2165-8048.20.10.314
 20. Wallace DC. A Mitochondrial Paradigm of Metabolic and Degenerative Diseases, Aging, And Cancer: A Dawn for Evolutionary Medicine. *Annu Rev Genet*, 2005; 39: 359-407.
 21. Ferrari R, Ceconi C, Curello S, Cargnoni A, Pasini E, Visioli O. The Occurrence of Oxidative Stress During Reperfusion in Experimental Animals and Men. *Cardiovasc Drugs Ther.*, 1991; 5(2): 277-87.
 22. Andersen JK. Oxidative Stress in Neurodegeneration: Cause or Consequence? *Nat Med.*, 2004; 10: S18-25.
 23. Ohta S, Ohsawa I. Dysfunction of Mitochondria and Oxidative Stress in The Pathogenesis of Alzheimer's Disease: On Defects in The Cytochrome C Oxidase Complex and Aldehyde Detoxification. *J Alzheimers Dis.*, 2006; 9: 155-66.
 24. Ohta S, Ohsawa I, Kamino K, Ando F, Shimokata H. Mitochondrial ALDH2 Deficiency as An Oxidative Stress. *Ann N Y Acad Sci.*, 2004; 1011: 36-44.
 25. Chang JC, Kou SJ, Lin WT, Liu CS. Regulatory Role of Mitochondria in Oxidative Stress and Atherosclerosis. *World J Cardiol*, 2010; 2: 150-9.
 26. Finkel T, Holbrook NJ. Oxidants, Oxidative Stress and The Biology of Ageing. *Nature*, 2000; 408: 239-47.
 27. Ohsawa I, Ishikawa M, Takahashi K, Et Al. Hydrogen Acts as A Therapeutic Antioxidant by Selectively Reducing Cytotoxic Oxygen Radicals. *Nat Med.*, 2007; 13: 688-94.
 28. Nicolson GL, De Mattos GF, Settineri R, Costa C, Ellithorpe R, Steven R, Et Al. Clinical Effects of Hydrogen Administration: From Animal and Human Diseases to Exercise Medicine. *Intern J Clin Med.*, 2016; 7: 32-76.
 29. Ohno K, Ito M, Ichihara M, Ito M. Molecular Hydrogen as An Emerging Therapeutic Medical Gas for Neurodegenerative and Other Diseases. *Oxid*

- Med Cell Long, 2012; 2012: 1-11.
30. Ohta S. Molecular Hydrogen as A Novel Antioxidant Hydrogen Sulfide in Redox BiolPart B., 2015; 555: 289–317.
 31. Iketani M, Ohsawa I. Molecular Hydrogen as A Neuroprotective Agent. *Curr Neuropharma*, 2017; 15(2): 324-331.
 32. Zhang J, Wu Q, Song S. Effect of Hydrogen-Rich Water on Acute Peritonitis of Rat Models. *Intern Immunopharm*, 2014; 21(1): 94-101.
 33. Wu Q, Zhang J, Wan Y, Song S, Zhang Y, Zhang G, Et Al. Hydrogen Water Alleviates Lung Injury Induced by One-Lungventilation. *J Of Surg Res.*, 2015; 199(2): 664-670.
 34. Current Progress in Molecular Hydrogen Medication: Protective and Therapeutic Uses of Hydrogen Against Different Disease Scenarios. Tarekegn Gebreyesus Abisso, Yao Mawulikplimi Adzavon. *Et. All, Intern Med.*, Jan-2020; 10: 314. Doi: 10.35248/2165-8048.20.10.314.
 35. Nakayama M, Nakano H, Hamada H. A Novel Bioactive Hemodialysis System Using Dissolved Dihydrogen (H₂) Produced by Water Electrolysis: A Clinical Trial. *NephrolDialysis Transpl*, 2010; 25(9): 3026-3033.
 36. Yach D, Hawkes C, Gould CL, Hofman KJ. The global burden of chronic diseases: overcoming impediments to prevention and control. *JAMA*, Jun, 2, 2004; 291(21): 2616-22. doi: 10.1001/jama.291.21.2616. PMID: 15173153.
 37. <https://www.who.int/data/stories/world-health-statistics-2021-a-visual-summary>
 38. Keith P. McCullough, Hal Morgenstern, Rajiv Saran, et.all. Projecting ESRD Incidence and Prevalence in the United States through 2030. *JASN*, Jan 2019; 30(1): 127-135. DOI: 10.1681/ASN.2018050531
 39. Liyanage T, Ninomiya T, Jha V, et.all Worldwide access to treatment for end-stage kidney disease: a systematic review. *Lancet*, May 16, 2015; 385(9981): 1975-82.
 40. Pizzino G, Irrera N, Cucinotta M, et al. Oxidative Stress: Harms and Benefits for Human Health. *Oxid Med Cell Longev*, 2017; 2017: 8416763. doi:10.1155/2017/8416763
 41. Hydrogen gas (XEN) inhalation ameliorates airway inflammation in asthma and COPD patients. Shao-Ting Wanga, Cheng Bao et. all. *QJM: An International Journal of Medicine*, 2020; 870–875. doi: 10.1093/qjmed/hcaa164.
 42. Therapeutic Efficacy of Molecular Hydrogen: A New Mechanistic Insight, Toru Ishibashi. *Current Pharmaceutical Design*, 2019; 25: 946-955. DOI:10.2174/1381612825666190506123038
 43. Ichihara M, Sobue S, Ito M, Ito M, Hirayama M, Ohno K. Beneficial biological effects and the underlying mechanisms of molecular hydrogen - comprehensive review of 321 original articles. *Med Gas Res.*, 2015; 5: 12. [<http://dx.doi.org/10.1186/s13618-015-0035-1>] [PMID: 26483953]
 44. Shirahata S, Kabayama S, Nakano M, et al. Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage. *Biochem Biophys Res Commun*, 1997; 234(1): 269-74.
 45. Kajiyama S, Hasegawa G, et.all, Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. *Nutr Res.*, Mar, 2008; 28(3): 137-43. doi: 10.1016/j.nutres.2008.01.008. PMID: 19083400.
 46. Ming, Y., Ma, Q. H., Han, X. L., & Li, H. Y. Molecular hydrogen improves type 2 diabetes through inhibiting oxidative stress. *Experimental and therapeutic medicine*, 2020; 20(1): 359–366. <https://doi.org/10.3892/etm.2020.8708>.
 47. Sugai, K., Tamura, T., Sano, M. et al. Daily inhalation of hydrogen gas has a blood pressure-lowering effect in a rat model of hypertension. *Sci Rep.*, 2020; 10: 20173. <https://doi.org/10.1038/s41598-020-77349-8>
 48. Dixon, Brandon J et al. “The evolution of molecular hydrogen: a noteworthy potential therapy with clinical significance.” *Medical gas research*, 16 May. 2013; 3(1): 10., doi:10.1186/2045-9912-3-10
 49. Sano, M., Suzuki, M., Homma, K., Hayashida, K., Tamura, T., Matsuoka, T., Katsumata, Y., Onuki, S. and Sasaki, J., Promising novel therapy with hydrogen gas for emergency and critical care medicine. *Acute Med Surg*, 2018; 5: 113-118. <https://doi.org/10.1002/ams2.320>
 50. Nakayama, Masaaki et al. “Possible clinical effects of molecular hydrogen (H₂) delivery during hemodialysis in chronic dialysis patients: Interim analysis in a 12-month observation.” *PloS one*, 12,9 e0184535. 13 Sep. 2017, doi: 10.1371/journal.pone.0184535
 51. Sai li, Rongrong liao et.all. Hydrogen Gas in Cancer Treatment. *Front. Oncol.*, 06 August 2019 | <https://doi.org/10.3389/fonc.2019.00696>.
 52. Promising novel therapy with hydrogen gas for emergency and critical care me