

Mitochondrial bioenergetics and oxidative balance in in vitro arbovirus infection models: a systematic review

Bioenergética mitocondrial e balanço oxidativo em modelos de infecções in vitro por arbovírus: uma revisão sistemática

Bioenergética mitocondrial y balance oxidativo en modelos in vitro de infecciones por arbovirus: una revisión sistemática

Received: 11/10/2022 | Revised: 11/28/2022 | Accepted: 11/30/2022 | Published: 12/07/2022

Wellington de Almeida Oliveira

ORCID: <https://orcid.org/0000-0002-0818-5543>
Universidade Federal de Pernambuco, Brazil
E-mail: wellington.braz96@gmail.com

Renata Emmanuele Assunção Santos

ORCID: <https://orcid.org/0000-0002-2166-3626>
Universidade Federal de Pernambuco, Brazil
E-mail: renataemmanuele@yahoo.com.br

Gizele Santiago de Moura Silva

ORCID: <https://orcid.org/0000-0003-4855-8639>
Universidade Federal de Pernambuco, Brazil
E-mail: gy.moura_13@hotmail.com

Kelli Nogueira Ferraz-Pereira

ORCID: <https://orcid.org/0000-0002-3180-0866>
Universidade Federal de Pernambuco, Brazil
E-mail: kellyferraz@hotmail.com

Ana Lisa do Vale Gomes

ORCID: <https://orcid.org/0000-0001-7067-1481>
Universidade Federal de Pernambuco, Brazil
E-mail: anavgomes@ufpe.br

Mariana Pinheiro Fernandes

ORCID: <https://orcid.org/0000-0002-9502-6355>
Universidade Federal de Pernambuco, Brazil
E-mail: mariana.fernandes@ufpe.br

Abstract

Introduction: Viral infections affect oxidative metabolism and may have repercussions on mitochondrial alterations, compromising cellular homeostasis. **Objectives:** To assess mitochondrial bioenergetics and oxidative balance in in vitro arbovirus infection models. **Methods:** The review was written in accordance with the PRISMA and submitted to the Open Science FrameWork platform with DOI 10.17605 / OSF.IO / 8ZF5W. Were used the Descriptors/MeSH (Arbovirus, Arboviruses, Arbovirus infections, Mitochondria, Oxidative stress and Reactive oxygen species) was carried out on the platforms: PubMed, SCOPUS, COCHRANE, Lilacs and Web of Science. The quality analysis of the studies was performed using the ARRIVE tool adapted to the CONSORT, followed by the KAPPA concordance test, were used 24 articles. **Results:** Results show morphological alterations in the mitochondria, such as swelling, fragmentation, and the appearance of membranes. Mitochondrial stretching was more intense in regions close to the convoluted zones, associated with changes in the genes of mitochondrial dynamics. Changes in oxidative stress biomarkers, antioxidant enzymes and ROS production were evident in most articles, except for those that used cells of immunological origin. **Conclusion:** Changes in mitochondrial bioenergetic can assist the virus in the replication process, however, these changes can result causing damage cell and of oxidative stress.

Keywords: Arboviruses; Cells; Mitochondria; Oxidative stress.

Resumo

Introdução: As infecções virais afetam o metabolismo oxidativo e podem repercutir nas alterações mitocondriais, comprometendo a homeostase celular. **Objetivos:** Avaliar a bioenergética mitocondrial e o balanço oxidativo em modelos in vitro de infecção por arbovírus. **Métodos:** A revisão foi escrita de acordo com o PRISMA e submetida à plataforma Open Science FrameWork com DOI 10.17605/OSF.IO/8ZF5W. Foram utilizados os Descritores/MeSH (Arbovirus, Arboviruses, Arbovirus infecções, Mitochondria, Oxidative stress and Reactive oxigênio species) foi realizado nas plataformas: PubMed, SCOPUS, COCHRANE, Lilacs e Web of Science. A análise da qualidade dos

estudos foi realizada por meio da ferramenta ARRIVE adaptada ao CONSORT, seguida do teste de concordância KAPPA, foram utilizados 24 artigos. Resultados: Os resultados mostram alterações morfológicas nas mitocôndrias, como inchaço, fragmentação e aparecimento de membranas. O estiramento mitocondrial foi mais intenso nas regiões próximas às zonas convolutas, associado a alterações nos genes da dinâmica mitocondrial. Alterações nos biomarcadores de estresse oxidativo, enzimas antioxidantes e produção de EROs foram evidentes na maioria dos artigos, exceto naqueles que utilizaram células de origem imunológica. Conclusão: Alterações na bioenergética mitocondrial podem auxiliar o vírus no processo de replicação, porém, essas alterações podem resultar em danos celulares e de estresse oxidativo.

Palavras-chave: Arbovírus; Células; Mitocôndria; Estresse oxidativo.

Resumen

Introducción: Las infecciones virales afectan el metabolismo oxidativo y pueden afectar los cambios mitocondriales, comprometiendo la homeostasis celular. Objetivos: Evaluar la bioenergética mitocondrial y el balance oxidativo en modelos in vitro de infección por arbovirus. Métodos: La revisión se escribió de acuerdo con PRISMA y se envió a la plataforma Open Science FrameWork con DOI 10.17605/OSF.IO/8ZFSW. Se utilizaron los Descriptores/MeSH (Arbovirus, Arboviruses, Infecciones por Arbovirus, Mitocôndrias, Estrés oxidativo y Especies de oxígeno reactivo) en las plataformas: PubMed, SCOPUS, COCHRANE, Lilacs y Web of Science. El análisis de la calidad de los estudios se realizó mediante la herramienta ARRIVE adaptada a CONSORT, seguida de la prueba de concordancia KAPPA, se utilizaron 24 artículos. Resultados: Los resultados muestran cambios morfológicos en las mitocondrias, como hinchazón, fragmentación y aparición de membranas. El estiramiento mitocondrial fue más intenso en las regiones cercanas a las zonas contorneadas, asociado con cambios en los genes de la dinámica mitocondrial. Los cambios en los biomarcadores de estrés oxidativo, las enzimas antioxidantes y la producción de ROS fueron evidentes en la mayoría de los artículos, excepto en aquellos que utilizaron células de origen inmunológico. Conclusión: Los cambios en la bioenergética mitocondrial pueden ayudar al virus en el proceso de replicación, sin embargo, estos cambios pueden resultar en daño celular y estrés oxidativo.

Palabras clave: Arbovirus; Células; Mitocôndrias; Estrés oxidativo.

1. Introduction

The incidence of arboviruses has come to be considered one of the main public health problems in recent years (Powers, 2017). According to the World Health Organization (WHO) (2014) (WHO, 2014), more than a billion people are infected, and more than a million people die every year from arboviruses. This index is associated with the diversity of arboviruses and the pathologies caused by them, as it is estimated that there are more than 500 species of arboviruses, among which, more than 100 are associated with diseases in humans (Lopes et al., 2014).

Among the diversity of arboviruses, some, such as Dengue, Zika, and Chikungunya stand out in this century for presenting the highest combined numbers of morbidity and mortality (Leta et al., 2014). In addition to these, yellow fever virus, Rift valley fever virus, Japanese encephalitis virus, Venezuelan equine encephalitis virus, and West Nile virus, also cause many infections every year. Most individuals infected with arboviruses are asymptomatic, but in symptomatic cases, symptoms can vary depending on the tropism of the virus by cells, organs, and systems. In mild and moderate cases of viral pathogenesis there may be the presence of fever, severe headaches, body aches, joint pain, vomiting, diarrhea, and rash (Azevedo et al., 2014). In more severe cases, other clinical manifestations may appear depending on the type of virus, such as, for example, meningitis or encephalitis, chronic arthralgia, hemorrhage, congenital malformations, and microcephaly, which can lead to death (Beckham & Tyler, 2014).

During infections, host cells can undergo modifications due to virus replication and infection, many of which are related to changes in cell metabolism (Maynard, 2010). One of the consequences of these changes induced by the virus in the metabolic pathways is the preference of infected cells to oxidize specific energetic substrates, such as, for example, the use of glucose for the replication of the virus, which can compromise cellular functions (Fontaine et al. 2014; Fernandes-siqueira et al., 2018). In addition, viral infections can also interfere with mitochondrial bioenergetics through effects on cellular respiratory functions and oxidative pathways (Fernandes-siqueira et al., 2018). These interferences include the altered redox state, dysregulation of energy metabolism, and changes in the structure and functions of the main complexes of the respiratory chain and mitochondrial

enzymes (Kim et al., 2014; Terasaki et al., 2012).

Mitochondria have several functions such as the detection of intracellular homeostasis disorders and regulation and transduction of signaling responses, especially during stress conditions (Tait et al., 2012). These organelles are responsible for the production of ATP, through the oxidative phosphorylation process, which occurs in the internal mitochondrial membrane through the respiratory chain, using oxygen as a substrate for consumption (Sheeran et al., 2017). During the transport of electrons through mitochondria, the formation of reactive oxygen species (ROS) also occurs, which in excess, when the action of antioxidant systems overlaps, can cause tissue damage and cell apoptosis (Pizzino et al., 2017). To combat excessive ROS production, the cell has antioxidant defense mechanisms (enzymatic and non-enzymatic). The enzyme system is composed mainly of the enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX), which have the function of reducing the levels of ROS, preventing oxidative stress (Birben et al., 2012).

In arbovirus infections, these mitochondrial functions can be affected, as shown in a study carried out with the Venezuelan equine encephalitis virus in which structural changes, excess ROS production, decreased mitochondrial membrane electrical potential, and induction of fission of the organelle were observed (Keck et al., 2017). In addition, other studies have already shown that viruses in the family Flaviviridae, such as dengue, (Olagnier et al., 2014) and West Nile virus (Gullberg et al., 2015) induced increased ROS production and oxidative stress in infected cells. In addition to these, another study carried out with CHIKV, a virus of the Togaviridae family, showed that the virus induces a decrease in the mitochondrial membrane potential and an increase in MDA levels. Knowing this, the current review proposes to evaluate mitochondrial bioenergetics and oxidative balance in in vitro arbovirus infection models.

2. Methodology

2.1 Search strategy

The research is a systematic review. The search was carried out without restriction of publication period and language. This systematic review was elaborated according to the items of the preferential reports for systematic analysis and meta-analysis (PRISMA) (Moher et al., 2009) found in table 4.

The search for articles was carried out in the following platforms: Medline / PubMed (Online System of Search and Analysis of Medical Literature), SCOPUS, COCHRANE, Lilacs (Health Sciences of Latin America and the Caribbean), and Web of Science. The following descriptors and MeSH were used in the platforms: Arbovirus, Arboviruses, Arbovirus infections, Mitochondria, Oxidative stress and Reactive oxygen species. The following crossings were performed: “Arbovirus Infections AND Mitochondria”, “Arboviruses AND Mitochondria”, “Arbovirus AND Mitochondria”, “Arbovirus AND Oxidative Stress”, “Arboviruses AND Oxidative Stress”, “Arbovirus Infections AND Oxidative Stress”, “Oxygen-reactive Species AND Arbovirus”, “Oxygen-reactive Species AND Arboviruses”, “Oxygen-reactive Species AND Arbovirus Infections”. The references cited by the articles were also reviewed to identify any possible additional publications. This review was submitted to the Open Science Framework with identification DOI 10.17605 / OSF.IO / 8ZF5W.

Table 4 - Guidelines PRISMA.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3-4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4-5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5-6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5-6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	*
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I ²) for each meta-analysis.	*

Source: Authors.

2.2 Eligibility criteria and exclusion criteria

After removal of duplicates, the full articles were analyzed when they met the following criteria: 1) Type of study, in vitro studies that did not carry out pharmacological intervention; 2) Studies that performed some analysis on mitochondrial bioenergetics and / or oxidative balance; 3) Studies that used an arbovirus for infection in cells; 4) Original studies.

Thus, the following were excluded from this research: Review articles, case reports, papers that did not address mitochondrial bioenergetics or oxidative balance, papers without comparison with control, and any other study that did not meet the eligibility criteria.

2.3 Methods for data extraction

Two reviewers independently extracted data from each included article. A pre-piloted and standardized form was used to extract data from the included studies to assess the quality and synthesis of evidence. The primary outcomes of the study were: oxidative stress biomarkers, mitochondrial morphology, activity of antioxidant systems, and production of ROS. Secondary outcomes were: type of virus, concentration of virus for infection, viral incubation period, and relationship of regression of infection over time.

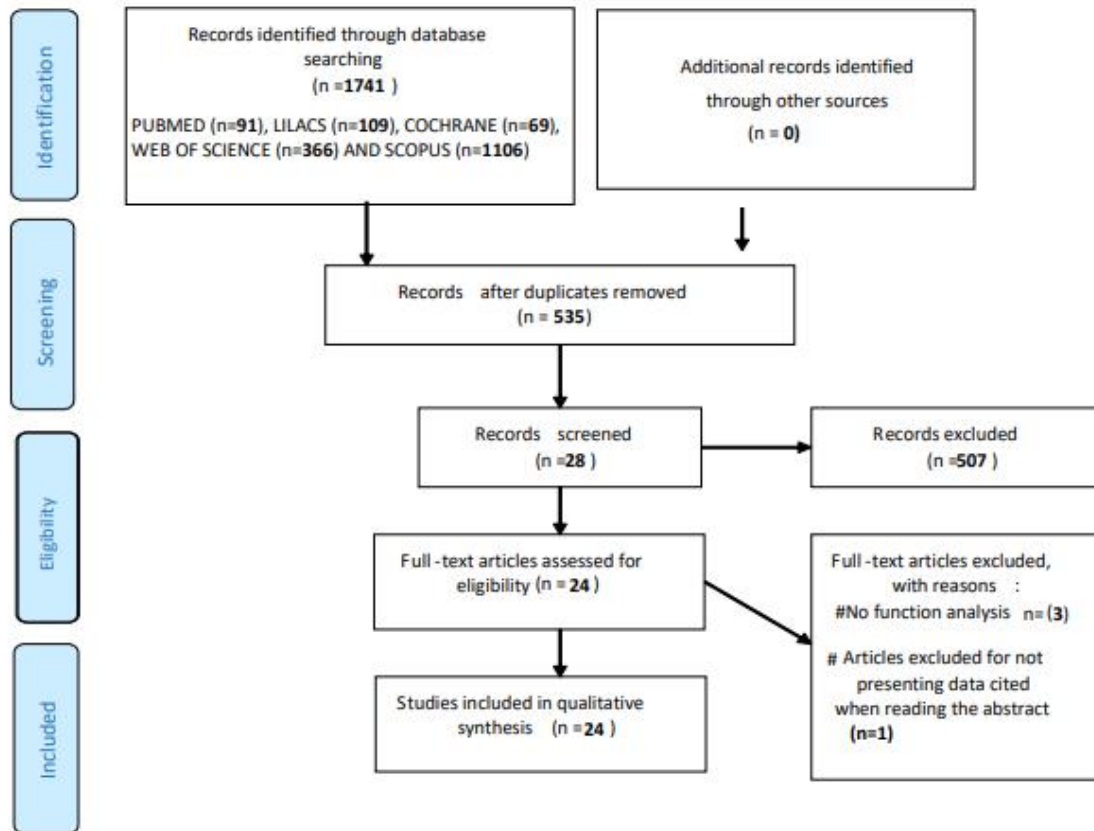
2.4 Quality assessment of included studies

In the absence of a consensus standard for evaluating the quality of in vitro studies, we used the modified ARRIVE tool, combined with the CONSORT guidelines (Consolidated standards for evaluation reports) for in vitro experiments, based on previous studies. The checklist contains 12 items to evaluate. For each item, a judgment related to the article was assigned, taking into account a pre-specified question. Next, Kappa statistics were used to assess interobserver agreement for items at risk of bias, using the software Statistical Package for the Social Sciences - SPSS version 20 for Windows (IBM SPSS Software, Armonk, NY, EUA). The results were synthesized in the form of summary tables and a descriptive summary to enable their comparison.

3. Results

After searching the electronic databases, 1741 articles were found. After the evaluation of duplicates, 1206 articles were excluded and 535 were submitted to reading of the title and abstract. In total, 28 articles were read in full because they fit the eligibility criteria, of which 4 were excluded due to the exclusion criteria. All results were published in English. The details of this search are shown in the Figure 1.

Figure 1 - Flowchart of the study selection process. The selection of studies took place in four phases:



Source: Authors.

3.1 Evaluation of the quality of studies

1) Identification: potential studies were collected from databases; 2) Screening: screening of the studies was carried out based on the reading of the titles and abstracts, according to the PICO scale; 3) Eligibility: studies were accepted according to the eligibility criteria for complete reading of the articles; 4) Articles selected after complete reading for data extraction and qualitative analysis.

The results of the Kappa tests revealed an almost perfect agreement, Kappa = 0.82 for the items evaluated by the tool (table 1) by described Viera, Garrett, 2005. The biggest disagreements were found in the objective items, sample discrimination, and reporting of procedures to reduce bias (Banerjee et al., 2018; Cherupanakkal et al., 2018; Cavalheiro Mariana et al., 2016; Camini et al., 2017; Dhanwani et al., 2012; Fernandes-siqueira et al., 2018; Keck et al., 2017; Keck et al., 2018; Lai et al., 2018; Moreno-altamirano et al., 2015; Mukherjee et al., 2013; Narayanan et al., 2011; Narayanan et al., 2014; Olagnier et al., 2014; Silva da Costa et al., 2012; Tung et al., 2010; Valero et al 2013; Verma et al., 2016; Yu et al., 2015). Regarding the objectives, some articles did not make their objective clear, with a tendency when writing the articles to observe the objectives implicit in the main results mentioned in the introduction. Regarding the discrimination of the samples, a failure was identified in the articles to detail the concentration of cells for the analyses, as well as the MOI (multiplicity of infection) of viruses that would be used for infection, which can represent a risk of bias when the analyses are associated with the evolution of the infection over a period. In addition, 13 of the 24 articles did not report any procedure to minimize possible bias in the study. Most of the articles presented a clear and adequate description of the results (n=17) and discussion (n=23) for the proposed study, indicating a good written

and argumentative relationship between the results and discussion, as well as clearly stating the values (mean, error, and standard deviation) of the results obtained.

3.2 Cell characteristics and virus titration used in the studies

The cells used in the studies, for the most part, were of human origin. The results show that 79% of the studies with in vitro virus infection used cells of human origin and the other 21% used neuronal cells from mice and / or rats. The diversity of cells of human origin was associated with the type of virus and its preference for the cell type. The majority of the studies, especially those with Flavivirus, used blood cells for the experiments (dendritic cells, monocytes / macrophages, lymphomononuclear cells). Regarding the concentration of cells used for the experiments, the lowest concentration used was 103cel / mL, (Keck et al., 2017) but most articles used the concentration of cells 106 cel / mL (Keck et al., 2017).

Regarding the virus titer, not all articles adequately defined the concentration used for the infection, but those which reported it presented an MOI variation from 0.1 to 10. The MOI value used in each experiment varied according to the cellular response to the virus infection, in general, an MOI of 2 or 5 was used in most articles. The studies were carried out with 5 families of pathogenic viruses in humans, covering the following families: Rhabdoviridae (n=1), Flaviviridae (n=13), Togaviridae (n=7), Peribunyaviridae (n=1), and Bunyaviridae (n=2).

Table 1 - Evaluation of studies selected in the research: Reporting of *In Vitro* Experiments (ARRIVE) guidelines.

Studies	List of evaluated items											
	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Item 11	Item 12
Mukherjee <i>et al.</i> , 2013	1	2	2	2	3	3	3	3	3	3	0	1
Narayanan <i>et al.</i> , 2011	1	3	2	2	2	2	2	3	2	1	1	1
Keck <i>et al.</i> , 2017	1	2	3	1	2	2	2	1	3	1	1	1
Fernandes-Siqueira <i>et al.</i> , 2018	1	2	2	3	2	3	2	3	2	1	1	1
Olagnier <i>et al.</i> , 2014	1	3	3	3	2	1	2	3	2	1	1	1
Dhanwani <i>et al.</i> , 2012	1	2	2	3	2	3	2	1	2	1	0	1
Yu <i>et al.</i> , 2015	1	2	3	3	2	3	1	3	2	1	1	1
Barbier <i>et al.</i> , 2017	1	2	2	1	2	2	1	2	3	1	1	1
Chatel-Chaix <i>et al.</i> , 2016	1	2	3	1	2	2	3	1	2	1	1	1
Moreno-Altamirano <i>et al.</i> , 2015	1	3	2	3	2	3	2	3	3	1	1	1
Datan <i>et al.</i> , 2016	1	3	3	1	2	3	2	1	2	1	1	1
Valero <i>et al.</i> , 2013	1	2	3	1	2	3	3	3	3	1	1	1
Qi y <i>et al.</i> , 2015	1	2	3	1	2	3	2	1	1	1	1	1
Lai <i>et al.</i> , 2018	1	2	2	3	2	2	3	3	3	1	1	1
Yang <i>et al.</i> , 2010	1	2	3	1	2	2	1	3	3	1	1	1
Tung <i>et al.</i> , 2010	1	3	3	3	2	2	1	3	3	1	1	1
Cherupanakkal <i>et al.</i> , 2018	1	2	2	3	2	2	1	3	3	1	1	1
Cavalheiro <i>et al.</i> , 2016	1	2	3	3	2	3	2	3	3	1	1	1
Verma <i>et al.</i> , 2016	1	3	3	3	2	3	3	3	2	1	1	1
Silva da Costa <i>et al.</i> , 2012	1	3	3	3	2	2	2	3	3	1	1	1
Keck <i>et al.</i> , 2018	1	3	2	3	2	3	3	3	3	1	1	1
Banerjee, Mukhopadhyay, 2018	1	3	3	3	2	2	1	3	2	1	1	1
Cmini <i>et al.</i> , 2017	1	2	2	3	2	3	3	3	3	1	1	1
Narayanan <i>et al.</i> , 2014	1	2	3	3	2	2	3	3	3	1	1	1

Legends: The signficante of the numbers: 1- Clearly inadequate, (2) Possibly accurate, (3) Clearly accurate. Source: Authors.

3.3 Arbovirus Infection and Mitochondrial Bioenergetics

The results of the studies show that arbovirus infections cause a series of mitochondrial changes that arise as a result of viral action or metabolic alterations in cells. To facilitate the understanding of the virus and mitochondria relationship, the results are described according to the family of the virus studied (Table 2).

In relation to the family Peribunyaviridae, infection by the La Cross virus, showed that in neurons there is an increase in the expression of the SARM1 binding protein and that this protein has a strong relationship with mitochondrial damage. The neurons infected by the virus showed mitochondria in a state of degeneration and increased production of EROS. The increase in the production of EROS was also identified in infections by viruses of the Togaviridae and Flaviviridae family.

With respect to the Flaviviridae family, in infected cells, the mitochondria in the host undergo changes in the consumption of energy substrate, for example, in DENV infections, mitochondria use palmitate and glucose as an energy substrate, whereas in non-infected cells glutamine is the most commonly used substrate. In addition, studies show that DENV infection increases the oxidation capacity of fatty acids. In another study (Chatel-Chaix et al., 2016) showed that in infected cells there is an increase in the mitochondrial respiration process, indicating greater availability of ATP for the cell, which contributes to viral replication (Chatel-chaix et al., 2016). Mitochondrial morphology also changes during Flavivirus infections, as the results indicate that the infection inhibits the expression of proteins associated with mitochondrial fusion and induces an increase in DRP1 expression. The increase in DRP1 expression associated with a decrease in MFN1 and MFN2 proteins induces a mitochondrial fission process. An increase in mitochondria elongation was also seen in some regions of the cell, especially near the convoluted membranes (CM), regions of intense viral replication during Flavivirus infection (Chatel-Chaix et al., 2016; Lai et al 2018; Fernandes-siqueira et al., 2018, Olagnier et al., 2014, Yu et al., 2015,).

Regarding the Togaviridae family, studies were found with MAYV, VEEV, CHIKV, and SINV viruses. Regarding MAYV, studies show that infected cells demonstrate an increase in the production of ROS after 6 hpi and that this production signals the immune system to produce TNF- α , indicating that there is a relationship between the immune response and the production of EROS. With respect to SINV, in intact cells, there is no change in O₂ consumption during 15hpi, but there is a reduction in the electron transport system (ETS); in permeabilized cells, however, there is a reduction, although not statistically significant, in the capacity of oxidative phosphorylation of cells when using substrates of complex I or II, of the electron transport chain. Thus, the capacity of the ETS dependent on CI and CII was not significantly affected after 15 h of infection by SINV, contrasting with the results with intact cells, where a significant decrease in maximum uncoupled respiration was observed at this time. However, the data point to a decrease in the activity of the mitochondrial membrane potential of cells infected by CHIKV and VEEV. Along with this change there is an increase in DRP1, indicating an increase in mitochondrial fission, in addition to changes in infected mitochondria such as swelling, membrane compression, and aggregation of damaged mitochondria (Cavalheiro Mariana et al., 2016; Dhanwani et al., 2012; Keck et al., 2017; Silva da Costa et al., 2012).

Table 2 - Results related to mitochondrial bioenergetics.

Author	Family of virus	Type Virus/MOI	Cell/Concentration	Objective	Results
Mukherj, <i>et al</i> (2013)	<i>Peribunyaviridae</i>	La Cross vírus (LACV)/0.01; 0.1 and 1	Primary Neuron/8.10 ⁵ cel/MI	To assess the relationship of SARM1 in neuronal apoptosis that is caused by LACV infections .	<ul style="list-style-type: none"> SARM1 protein is associated with mitochondrial damage; Infected cells showed an increase in the expression of SARM1 binding proteins with the mitochondria. Neurons containing swollen and / or damaged mitochondria in cell bodies and axons from cultures infected with LACV; Blocking the mitochondrial antiviral signaling protein (MAVS), showed an inhibition of SARM1 activity and EROS production. There is no blockage of MAVS when there is an increase in the production of EROS; SARM1 interacts with MAVS and induces oxidative stress, mitochondrial damage, and neuronal death.
Fernandes-siqueira <i>et al</i> (2018)	<i>Flaviviridae.</i>	Dengue vírus (DENV) / MOI of 1	Huh7 cells, a strain of human hepatocarcinoma / The cells were seeded in 60 cm plastic Petri dishes, at a density of 10 ⁵ cel / mL, but in experiments such as oxygen consumption, concentrations of 10 ⁶ cel / mL were used	Using high-resolution respirometry to assess metabolic circuits that occur during DENV infection	<ul style="list-style-type: none"> Infection of cells by DENV inhibits mitochondrial breathing; Infected Huh7 cells effectively use palmitate as an oxidative substrate, since oxygen consumption in the presence of palmitate was higher than that observed in the control; Glutamine was the best substrate used by control cells in all conditions, however, DENV infection strongly inhibited the use of this nutrient as an energy substrate for cells; The presence of glucose in the control cells inhibited consumption, but these effects were attenuated in cells infected by DENV, confirming that the use of glucose by these cells increases the oxidation capacity of endogenous fatty acids.
Olagnier <i>et al</i> (2014)	<i>Flaviviridae</i>	Dengue vírus (DENV)/ not described	Dendritic cells derived from primary human monocytes (Mo-DC) / 10 ⁶ cel/mL	To conduct an in-depth analysis of the transcriptome, along with biochemical and functional analyses of the early host response to DENV infection in Mo-DC	<ul style="list-style-type: none"> The number of mitoSOX positive cells increased from 31.9 ± 12.3% (uninfected) to 56.8 ± 7.9% (infected) after 48 hours of infection; Both the mitochondrial release of ROS and mitochondrial depolarization were associated with the induction of apoptosis, through the mitochondria-dependent pathway in Mo-DC cells infected with DENV.
Yu CY <i>et al</i> (2015)	<i>Flaviviridae</i>	DENV/ MOI of 10	Cells A549 / mitoYFP and A549 / mitoCherry	To evaluate, in DENV infections, how the mitochondrial dynamics behave	<ul style="list-style-type: none"> In simulation-infected cells, continuous mitochondrial fusion / fission led to uniform redistribution and colocalization of mitoYFP and mitoCherry (mitochondrial markers) in fused cells, while much less colocalization of mitoYFP and mitoCherry was observed in DENV infected cells within 24 days after infections, but without significant cytotoxicity. The levels of proteins related to fission, Drp1, pDrp1, optic atrophy 1 (OPA1) and mitochondrial fission 1 (Fis1) were similar during the course of infection, while MFN1 and MFN2 proteins were reduced in infected cells; In cells that had induction of MFN1 overexpression, the proportion of cells with clustered mitochondria decreased from 86.2% to 37.1% when compared to cells simulated to those infected with DENV; It has been suggested that DENV can block MFN1-mediated mitochondrial fusion.
Barbier <i>et al</i> (2017)	<i>Flaviviridae</i>	Dengue vírus (DENV) / Not described	Vero cells, reporter cells (Huh7.5), and human liver cancer cells (HepG2) / Not described	To evaluate the relationship of Drp1 with mitochondrial length and respiration during DENV infection.	<ul style="list-style-type: none"> Using images of live cells from cultures infected with DENV, it was seen that the infection induces mitochondrial elongation; The mitochondria of cells infected with DENV showed increased respiration, which can lead to an increase in ATP production, when compared to uninfected cells;

					<ul style="list-style-type: none"> • Viral proteins, such as NS4b, were preferentially located in subcellular fractions of mitochondria; • The characterization of fusion and fission proteins revealed a decrease in Drp1 in mitochondria in the Huh7 cell line and in monocyte-derived dendritic cells (DCs); • The decrease in Drp1 was correlated with a reduction in the phosphorylation of Drp1 in S616 in the Huh7 cell line. On the other hand, the induction of mitochondrial fission or overexpression of Drp1 and Drp1 S616D of wild type (WT) inhibited DENV replication.
Chatel-Chaix. <i>et al.</i> , 2016	<i>Flaviviridae</i>	Dengue vírus (DENV) / Not described	Huh7 cells / 1.10^5 cel/mL	To evaluate the function of NS4B protein during DENV infection	<ul style="list-style-type: none"> • Mitochondria were elongated and often in physical contact with punctuated structures that contained NS3 and NS4B; • In cells infected with DENV, the elongation of the mitochondria became visible for the first time 23 hours after infection (hpi) and remained between 35 and 50 hpi; • The development of infection in the mitochondrial network collapsed, concomitantly with cell death due to cytopathic effects induced by DENV; • Elongation of the mitochondria has been observed in cells infected with DENV2; • The greatest mitochondrial elongation was found close to the convoluted membranes (CM) of the infected cells, which are sites of greater viral replication; • A large spatio-temporal relationship was detected between the increase in CM and mitochondrial elongation, where cells with CMs were detectable at 21 hours after infection and accumulated over time, and 86% of cells with CMs showed a network of elongated mitochondria. In approximately 36% of infected cells, it was seen that the formation of CMs and the elongation of mitochondria occurred simultaneously; • The decrease in the expression of DRP1 stimulated the mitochondria elongation, while the reduced expression of MFN2 favored its fragmentation, without affecting cell viability during a 5-day observation period; • The elongation mediated by the decrease in DRP1 stimulated the replication of both strains of DENV (DVs and NGC), while the fragmentation of mitochondria forced by the decrease in MFN2 inhibited DENV replication, suggesting a relationship of viral replication capacity and expression of mitochondrial dynamics proteins.
Lai et al (2018)	<i>Flaviviridae</i>	Dengue vírus (DENV) / MOI of 5	Dendritic cells (DC) and Human lung epithelium cell (A549) / DC: 10^6 cel / mL and A549: $2,5 \times 10^5$ cel/mL	To evaluate the role of the TLR9 gene (specific mitochondrial DNA receptor) in DENV infection	<ul style="list-style-type: none"> • The total number of cell mtDNA copies decreased slightly after DENV infection within 24 hours; • There was a significant and proportional increase in cytosolic mtDNA 12 and 24 hours after DENV infection; • DENV-induced TLR9 activation and mtDNA release were also demonstrated when DCs were infected with different DENV serotypes (serotypes 1, 3, and 4), as well as different DENV2 strains; • DENV infection induced an increase in ROS in DCs, evidenced by increased staining with MitoSOX. These effects were inhibited by MitoTEMPO, a mimetic of superoxide dismutase directed at mitochondria, which has properties for eliminating superoxide and alkyl radicals; • Treatment with MitoTEMPO reduced the total oxidized DNA and, specifically, the levels of cytosolic oxidized DNA; • MitoTEMPO inhibited the release of mtDNA induced by DENV in the cytosol and expression of TLR9; • EROS production induces the release of mtDNA that activates TLR9 expression.
Cavalheiro et al (2016)	<i>Togaviridae</i>	Mayaro vírus (MAYV) / MOI of 1 to 5	Macrophages/ 10^6 cel/mL	To investigate MAYV's ability to replicate in macrophages and to induce inflammatory responses and cell death	<ul style="list-style-type: none"> • MAYV induces a progressive increase in the generation of ROS in the early stages of infection (6 h), with intense viral replication before the peak of TNF production and cell death. • Investigation of the role of ROS in the production of virus-induced TNF showed a reduction in the release of TNF by infected cells to baseline levels, indicating an involvement of ROS signaling in the release of inflammatory cytokines by macrophages during MAYV infection.
Silva da Costa et al (2012)	<i>Togaviridae</i>	Sindbis vírus (SINV) / MOI of 1 to 5	Mouse neuroblastoma (Neuro 2a) / 10^6 cel/mL	To characterize morphologically and biochemically, and evaluate mitochondrial bioenergetics and energy homeostasis in SINV infections	<ul style="list-style-type: none"> • The routine breathing of Neuro 2a cells (in culture medium containing 5 mM glucose) did not differ between infected and simulation; • The rate of oxygen consumption in the presence of oligomycin in cells with simulated infection was 31.1 and that of cells infected with SinV was 26.6 pmoles. There was a statistical tendency for leakage breathing to decrease after 15 h of SinV infection;

					<ul style="list-style-type: none"> The capacity of the electron transport system (ETS) was reduced by 36% in Neuro 2a cells after 15 h of infection by SinV, while the average rates in the infected simulations and SINV-infected Neuro 2a cells were 139.6 and 102.7 pmoles; After 24 h the infected cells cleared, so that the same fraction of ETS was activated to conduct ATP synthesis; Regarding permeabilized cells, after 15 h of infection, there was a reduction, although not statistically significant, of the oxidative phosphorylation capacity of Neuro 2a cells supported by Complex I (CI: pyruvate / malate) or Complex II (CII) substrates: succinate). The maximum uncoupled breath measured after adding the FCCP did not differ in infected and infected cells by simulation. Therefore, the capacity of the ETS depending on CI and CII was not significantly affected after 15 h of infection by SinV, contrasting with the results of intact cells, where a significant decrease in maximum uncoupled respiration was observed at this time.
Keck f et al (2018)	<i>Togaviridae</i>	Venezuelan Equine Encephalitis Virus (VEEV) / MOI of 2 to 10	Human astrocytoma cells (U-87 MG), VERO cells, and murine microglial cells (BV2) / U-87MG and BV2	To determine whether murine microglia is susceptible to VEEV infection and whether the infection results in mitochondrial dysfunction	<ul style="list-style-type: none"> Treatment with BAY-82 and MitoQ not only recovered the electrical potential of the mitochondrial membrane (MMP) in the infected cells, but also increased this electrical potential in the treated samples compared to the uninfected simulated control. In their most effective form, BAY-82 and MitoQ increased the membrane potential by 29% and 48%. The antioxidant directed at mitochondria is more effective in restoring mitochondrial function than the anti-inflammatory BAY-82.
Dhanwan i R et al (2012)	<i>Togaviridae</i>	Chikungunya vírus (CHIKV) / MOI of 0.1 to 5	Human neuroblastoma (SH-SY5Y) /1.10 ⁶ cel/mL	To evaluate the role of apoptosis and oxidative damage in SH-SY5Y cells infected with CHIKV	<ul style="list-style-type: none"> The results indicated a significant change in the electrical potential of the mitochondrial membrane (MMP). JC-1 staining of infected cells revealed a change in fluorescence from red to green, consistent with a decrease in mitochondrial membrane potential
Keck, f et al (2017)	<i>Togaviridae</i>	Venezuelan Equine Encephalitis Virus (VEEV)/ MOI of 2 to 10	Human astrocytoma cells (U87MG cells) and African green monkey kidney cells (Veros) / Concentration varied according to the average tests used/ 10 ³ cel/mL	To assess the role of mitochondrial dynamics in VEEV infections	<ul style="list-style-type: none"> TC-83 infection resulted in a decrease in membrane potential at all times tested, with significant reductions of 6 hpi in the highest MOIs (10 and 20) and in all MOIs tested at 24 hpi when compared to the respective controls simulated. The infected group showed an increase in the expression of the Drp1 protein from 24 dpi in MOI of 5 to 10, while in MOI of 20 there was a drop in levels. Infected cells showed a series of changes in structure, including partially swollen mitochondria, inner membrane compaction, and assembled mitochondria.

Abbreviations: Moi (multiplicity of infection); dpi (days after infection); hpi (hours after infection). Source: Authors.

3.4 Oxidative balance and Arbovirus infection

Oxidative balance is extremely important for maintenance of cellular homeostasis. The results show several changes in the levels of oxidative stress biomarkers, and expression and/or antioxidant enzymes activity, in addition to changes in the cellular redox state. Changes were verified in the ROS production in more than 80% of the articles of this review. All the results described with respect to oxidative balance are shown in Table 3.

3.4.1 Antioxidant enzymes and oxidative stress biomarkers

In the Bunyaviridae family, the results show that in infections with Rift Valley Fever Virus (RVFV), SOD activity decreases in infected cells, and when the SOD is blocked, apoptosis levels increase about seven times (Narayanan et al., 2011). These results are similar to those of Banerjee and Mukhopadhyay (2018) (Banerjee et al., 2018), who observed a decrease in SOD activity in Chikungunya virus (CHIKV) infections, but differ from those found in the Flaviviridae family, where Valero et al. (2013) (Valero et al 2014), showed an increase in SOD activity in Dengue virus (DENV) infections. In the Togaviridae family, a study conducted with Mayaro virus (MAYV) showed an increase in SOD activity throughout the period of infection. In addition, in the Bunyaviridae family a negative regulation was observed between the SOD levels and ROS production (11).

Regarding catalase, no studies have evaluated its activity in the family Bunyaviridae and Rhabdoviridae. However, studies with the Flaviviridae and Togaviridae families show that Arbovirus infection results in increased catalase activity associated with the proportion of virus per cell (Camini et al., 2017; Valero et al., 2013).

The articles that assessed GSH levels were performed with viruses from the families Flaviviridae and Togaviridae. The results show that in DENV and MAYV infections, there is an increase in GSH levels, however, in cells infected with CHIKV there was a decrease in GSH, associated with an increase in the time of infection. The GSH/GSSG ratio was performed in only one article, in which a decrease in the cell REDOX status between 15 to 24 hpi was observed in MAYV infections (Camini et al., 2017; Valero et al., 2013).

Some articles also evaluated MDA levels in infected cells. For Flavivirus infections, Cherupanakkal et al (2018) (Cherupanakkal et al., 2018) showed that in lymphomonuclear cells, DENV infection did not cause changes in MDA levels, however when related to cell apoptosis there was a significant difference between the infected group and the control group. In contrast, Valero et al (2013) showed that monocytes infected by DENV show increased MDA levels (Valero et al 2013). These discrepancies may be associated with the methodology used to infect the cells depending on the strain type. Regarding the Togaviridae family, Banerjee and Mukhopadhyay (2018) showed that in infections by the Chikungunya virus, lymphomonuclear cells of patients with or without polyarthralgia have high levels of MDA and high levels of the MDA/SOD ratio, indicating a possible situation of oxidative stress in these cells (Banerjee et al., 2018).

3.4.2 ROS and NO production

The ROS production was evaluated in most studies, thus, the ROS levels of all virus families in this review were quantified. Only one article did not show an increase in intracellular ROS levels; in that article the neutrophils infected by DENV with an MOI of 10 did not demonstrate an increase in the ROS production. In addition, the author concluded that the production and neutrophil activity are not associated with ROS (Moreno-altamirano et al., 2015).

In the Bunyaviridae family, there was an increase in the ROS production in liver cells infected by RVFV, associated with the presence of non-structural viral proteins and a decrease in SOD levels, where the author reports that there is down-regulation between the decrease in SOD levels and increase in ROS (Narayanan et al., 2011). In the family Flaviviridae, the ROS increase was found in infections by DENV and JEV, in several cell types. In addition, astrocytes isolated from rats and infected by JEV showed a relationship between the increase in ROS and the expression of metalloproteinase-9 (mmp-9) (Cherupanakkal

et al., 2018; Datan et al., 2016; Qi et al., 2015; Tung et al., 2010; Valero et al 2013; Yang et al., 2010). Studies with Flavivirus have shown that an increase in ROS occurs between 18 to 48 hpi. The Togaviridae and Rhabdoviridae families also demonstrated increases in ROS in infections by VEEV, CHIKV, SINV, and CHPV (Keck et al., 2018; Verma et al., 2016).

Regarding NO production, two studies carried out experiments to quantify NO production. In the first study performed with DENV, it was observed that in infections by the 4 dengue serotypes there is an increase in NO production, however the highest production was found in DENV-2. In addition, the NO increase was associated with an increase in MDA (Valero et al 2013). The other study was carried out with CHPV, in which Verma et al. (2016) showed that at 16hpi there is an increase in NO production associated with ROS production in BV-2 cells (Verma et al., 2016). In addition, when the neuronal apoptosis index was evaluated, an increase in the cell death of the infected group was evidenced, indicating that NO and ROS production may be associated with apoptosis of infected cells.

Table 3 - Oxidative balance in arbovirus infections.

Author	Family of virus	Type Virus/MOI	Cell/Concentration	Objective	Results
Narayanan <i>et al.</i> , 2011	<i>Bunyaviridae</i>	Rift Valley Fever Virus (RVFV) / The viruses of the MP12 strain were infected at an MOI of 3, while the viruses of the ZH501 strain at an MOI of 2	Lung epithelial cells of small human airways (HSAECs)/ 1.10 ⁶ cell/mL	To determine if RVFV infection caused oxidative stress in infected human cells and if there were host responses associated with this condition of cellular stress and production of tumor necrosis factor alpha (TNF- α)	<ul style="list-style-type: none"> • The infected cells showed a consistent decrease in expression of superoxide dismutase 1 (SOD1) from the initial moments (24 and 30 h); • There was an increase in the ROS production in infected cells related to the negative regulation of cytosolic SOD; • The increase in the production of superoxides was visible from 12dpi and 24dpi; • Control cells treated with TNF-α showed lower levels of SOD1 compared to untreated cells, in a 24-hour period; • After depletion of SOD1, the percentage of cells undergoing apoptosis was almost seven times higher than the control cells; • SOD1-depleted cells that were not infected by the virus had significantly less apoptosis (1.5 times greater than control cells; data not shown); • The results indicate that SOD1 depletion makes the cells more susceptible to apoptosis during MP12 infection; • Activation of the AMPK pathway was necessary to combat the damage caused by changes in the SOD1 concentration; • Cells infected with the ZH501 strain of RVHV were subjected to the same experiments and demonstrated similar results, but the MOI used was lower than that of the MP12 strain due to its pathogenicity.
Narayanan <i>et al</i> (2014)	<i>Bunyaviridae</i>	Rift Valley Fever Virus (RVFV) / MOI of 3.5 or 10	Liver stellate cell line (CFSC-8B) / 1.10 ⁶ cell/mL	To investigate whether oxidative stress occurs and whether it leads to apoptotic responses in patients infected with RVFV	<ul style="list-style-type: none"> • Quantification of fluorescence revealed an approximate 6-fold increase in superoxide levels in virus-infected cells over controls infected by simulation; • The presence of the viral protein NSs in the mitochondria of infected cells contributes to the early increase of ROS; • ROS increased levels correlated with the activation of nuclear factor-κB (NFκB), p65 and p53 responses, which together with the infection were also reflected as macromolecular rearrangements; • ROS act as critical contributors to liver cell apoptosis during RVFV infection.
Moreno-Altamirano <i>et al</i> (2015)	<i>Flaviviridae</i>	Dengue Virus (DENV) / MOI of 10	Neutrophils / 10 ⁶ cell/mL	To assess whether Nets (extracellular neutrophil traps) are released by DENV2-infected neutrophils	<ul style="list-style-type: none"> • There was no change in the ROS production in DENV infections, thus, the production and activity of neutrophils is not associated with ROS production;

Yang T et al (2010)	<i>Flaviviridae</i>	Japanese encephalitis virus (JEV)/MOI 0.1 to 1	Human lung epithelial cells (A549) / 5.10 ⁶ cell/mL	To identify apoptotic processes and pathways related to JEV infection	<ul style="list-style-type: none"> • The ROS production in JEV infected cells increased tenfold 24 and 48 hours post-infection; • The ROS increase signaled cell apoptosis and correlated with negative regulation of thioredoxin in infected cells.
Olagnier et al (2014)	<i>Flaviviridae</i>	Dengue virus (DENV)/ Not described	Primary human monocyte-derived dendritic cells (Mo-DC) / 10 ⁶ cell/mL	To perform an analysis of the transcriptome, along with biochemical and functional analyses of the host's early response to DENV infection in Mo-DC	<ul style="list-style-type: none"> • The ROS production was observed in Mo-DC infected by DENV, with a 2-fold increase in DCF fluorescence detected by cytometry, 18 h after infection; • DENV infection increased the intracellular accumulation of ROS in a dose dependent manner.
Datan et al (2016)	<i>Flaviviridae</i>	Dengue virus (DENV) / MOI of 5	Canine renal cells Madin-Darby (MDCK) / 10 ⁴ cell/mL	To evaluate the mechanism of autophagy in DENV infection at an early stage and how this process is related to the endoplasmic reticulum (ER) and the protein kinase of the type R endoplasmic reticulum (PERK)	<ul style="list-style-type: none"> • The inhibition of ER stress or ataxia mutated telangiectasia (ATM) signaling cancels the protection provided by dengue against other cellular stressors; • Direct inhibition of the ER stress response in infected cells decreases the renewal of the autophagosomes, reduces the ROS production, and limits the dengue virus reproduction; • Blocking ATM activation, which is an early response to infection, decreases the transcription of ER stress response proteins, but the ATM has a limited impact on the ROS production and viral titers; • The ROS production determines only late-onset autophagy in infected cells and is not necessary for dengue-induced protection against stressors; • Among the various autophagy-inducing pathways during infection, ER stress signaling is most important for viral replication and cell protection.
Valero et al (2013)	<i>Flaviviridae</i>	Dengue virus (DENV-1, DENV-2 and DENV-4) / MOI of 1	Monocytes / 3.10 ⁵ cell/mL	To analyze the oxidative (nitric oxide-NO and malondialdehyde-MDA levels) and antioxidant (catalase-CAT, superoxide dismutase-SOD and reduced glutathione-GSH) responses of monocytes of newborns, young adults, and older adults during in vitro study of dengue virus infection	<ul style="list-style-type: none"> • Lower values of SOD, MDA, GSH, and NO were found in neonates and older adults, whereas the highest values were observed in adults; • The increase in NO, MDA production, catalase and SOD activities, and the GSH content were induced by different viral serotypes and LPS in monocytes of neonatal individuals, adults, and older adults; • DENV-2 induced the highest NO production accompanied by high MDA production; • The antioxidant response was observed in all types of DENV; • The highest values of catalase activity were observed for DENV-1 and DENV-4 and SOD activity for DENV-1; • The highest GSH content was observed in DENV-4 infected monocytes in all groups analyzed;

					<ul style="list-style-type: none"> The degree of oxidative and antioxidant response was influenced by the source of monocytes.
Qi Y et al (2015)	<i>Flaviviridae</i>	Dengue virus (DENV-2) / MOI of 4	Primary isolates from human umbilical veins (HUVECs) / 10 ⁶ cell/mL	To evaluate the interferon 6 (IFN-6) role in infection by DENV-2	<ul style="list-style-type: none"> The decrease in mitochondrial membrane potential (MMP) was noticeable from 24 hours to 48 hours after DENV2 infection; Infected IFN 6 cells (+/+) showed no changes in MMP for long periods of time. On the other hand, IFN6 -/- cells appeared to be more sensitive to DENV-2.
Tung et al (2010)	<i>Flaviviridae</i>	Japanese encephalitis virus (JEV) / Not described	Rat brain astrocytes (RBA-1)/Not described	To evaluate the molecular mechanisms underlying JEV-induced MMP-9 expression in RBA-1 cells	<ul style="list-style-type: none"> Treatment with JEV induced a significant increase in ROS levels, measurable in 5 minutes and sustained for more than 90 min, which may be associated with an increase in NADPH oxidase; JEV-induced MMP-9 expression was mediated through ROS-dependent activation of p42 / p44 MAPK in RBA-1 cells.
Cherupanakkal et al (2018)	<i>Flaviviridae</i>	Dengue virus (DENV) / Not described	Lymphomonuclear cells /Not described	To assess biomarkers of oxidative stress, apoptosis, and DNA damage in individuals infected by dengue virus	<ul style="list-style-type: none"> Increased levels of lipid peroxidation were found in the infected group when compared to controls with febrile illnesses and controls without febrile illnesses; When MDA data were correlated with apoptosis levels, a positive correlation was found according to Spearman's correlation.
Keck et al (2017)	<i>Togaviridae</i>	Venezuelan Equine Encephalitis Virus (VEEV) / MOI of 2 at 10	Human astrocytoma cells (U87MG) and African green monkey kidney cells (Veros) / 10 ³ cell/mL	To evaluate mitochondrial dynamics and ROS production in VEEV infections	<ul style="list-style-type: none"> The data indicated a significant ROS increase in cells infected with TC-83 compared to uninfected cells, in all MOIs and time intervals tested; U87MG cells treated with hydrogen peroxide (H₂O₂) demonstrated ROS accumulation.
Banerjee N, Mukhopadhyay (2018)	<i>Togaviridae</i>	Chikungunya virus (CHIKV) / Not described	Lymphomonuclear cells /Not described	To evaluate the ROS concentration, oxidative stress markers, antioxidant potential, and cytokines in the peripheral blood of patients infected by CHIKV	<ul style="list-style-type: none"> The MDA level was high in infected patients who did not have persistent polyarthralgia (ChikWoPP) when compared to healthy controls; Nitric oxide (NO) was 1.82 times higher in infected patients who had persistent polyarthralgia (ChikWPP); The ChikWopp group had higher levels of thiols than ChikWPP; Carbonyl levels were higher in patients with polyarthralgia; SOD levels were lower than the control; The SOD level was 1.02 times lower, while the radical scavenging activity was 1.16 times lower in infected patients than in controls; The MDA/SOD ratio was 1.57 times higher in the ChikWPP group; There was a 2.42-fold increase in intracellular ROS production when compared to the control group

Silva da Costa et al (2012)	<i>Togaviridae</i>	Sindbis virus (SINV) / MOI of 1 at 5	Neuroblastoma of mice (Neuro 2a) / 10 ⁶ cell/mL	To characterize morphologically and biochemically, evaluate mitochondrial bioenergetics and energy homeostasis in SINV infections	<ul style="list-style-type: none"> • After 15 h of infection, there was a 40% increase in the ROS accumulation when compared to uninfected cells; • ROS accumulation increased significantly (2.3-fold increase) after 24 hours of SINV infection, suggesting that ROS could have contributed to mitochondrial dysfunction and cell death of Neuro 2a induced by SINV.
Keck et al (2018)	<i>Togaviridae</i>	Venezuelan Equine Encephalitis Virus (VEEV) / MOI of 2 at 10	Human astrocytoma cells (U-87 MG), VERO cells and murine microglial cells (BV2) /U-87MG and BV2 10 ⁴ cell/mL, VERO 10 ⁵ cell/mL	To determine whether murine microglia is susceptible to VEEV infection and whether the infection results in mitochondrial dysfunction	<ul style="list-style-type: none"> • At 24 hpi using an MOI of 2, the ROS levels increased 256% and 171% in U-87 MG and BV2 cell lines, respectively; • The ROS increase corresponded to a decrease in the mitochondrial membrane potential (MMP) in both cell lines; • At 24 hpi, astrocytes infected with TC-83 exhibited a 53.6% decrease of the MMP compared to uninfected control; • Correlating with the lower ROS level, the BV2 microglia suffered a 40% decrease in MMP at 24 hpi; • The ROS accumulation and the MMP decrease were not dependent on the MOI and, therefore, all subsequent experiments were conducted using the MOI of 2; • The anti-inflammatory BAY-82 decreased the total ROS levels by 16% at 6 hpi and 22% at 24 hpi when compared to the control infected by TC-83.
Cmini FC et al (2017)	<i>Togaviridae</i>	Mayaro virus (MAYV) / MOI of 5	Human hepatocarcinoma cells (HepG2) or Macrophages (J774) / 2.5.10 ⁴ cell/mL or 1.10 ⁶ cell/mL	To investigate whether MAYV causes oxidative stress in infected cells and affects the host's antioxidant system	<ul style="list-style-type: none"> • There was an increase in total SOD activity at all times in cells infected with MAYV; • There was an increase in the CAT activity at 15 hpi, in cells infected with MAYV; • The total glutathione content in cells infected with MAYV increased by approximately 4 and 2.5 times at 6 and 15 hpi; • Total glutathione levels remained normal in cells at 24 hpi; • There was a progressive decrease in the GSH/GSSG ratio in HepG2 cells after infection by MAYV, with 35% and 37% reductions in this ratio at 15 and 24 hours after infection, respectively; • Similar to observations in HepG2 cells, ROS production was increased in MAYV-infected J774 cells; • Regarding HepG2 cells, there was an increase in ROS generation at all times tested (1, 2, 4, 6, 15, and 24 h pi), while in J774 cells an increase was observed after longer periods of infection (6, 15, and 24 h pi);

					<ul style="list-style-type: none"> • J774 cells showed a decrease in total SOD activity and increase in MDA levels at 6, 15, and 24 hpi
Dhanwani R et al (2012)	<i>Togaviridae</i>	Chikungunya virus (CHIKV) / MOI of 0.1 to 5	Human neuroblastoma (SH-SY5Y) / 1.10^6 cell/mL	To evaluate the role of apoptosis and oxidative damage in SH-SY5Y cells infected with CHIKV	<ul style="list-style-type: none"> • There was a decline in the GSH level over time; • Substances reactive to thiobarbituric acid (TBARS) production reached a maximum at 48 hpi. In addition, the oxidative stress level increased in parallel with the viral titer, at all times.
Verma AK et al (2016)	<i>Rhabdoviridae</i>	Chandipura virus (CHPV) / MOI of 0.1 for 2h	Rat hippocampal neuronal cell line (HT-22 cells) and mouse microglial cell line (BV2 cells) / 3.10^3 cell/mL	To evaluate the pattern of microglial activation and dosage of pro-inflammatory cytokines in CHPV infection	<ul style="list-style-type: none"> • The ROS and NO levels were evaluated at different incubation times and increased concomitantly with time; • It was found that the NO level was higher at 16 hpi; • The ROS level in the sample infected by CHPV was higher at 24 hpi, compared to those infected by simulation; • Regarding cell death, neuronal cells treated with the supernatant obtained from CHPV-infected microglia showed a greater number of TUNEL positive cells, compared to cells infected by simulation.

Abbreviations: MOI (multiplicity of infection); dpi (days after infection); hpi (hours after infection). Source: Authors.

4. Discussion

Viral infections have already been associated with imbalances in the oxidative metabolism of infected people, however, to date, no review has been carried out to identify how arbovirus infections act on REDOX status and mitochondrial activity in *in vitro* models. Therefore, the current study aimed to evaluate how arbovirus infections affect oxidative balance and mitochondrial bioenergetics in *in vitro* study models. Thus, analyzing the effects that infections cause in *in vitro* models, can serve as a basis for understanding the pathological mechanisms caused by arboviruses and directing pharmacological or non-pharmacological treatments to act more effectively in minimizing cell damage caused by infections.

4.1 Cell response to arbovirus infection

In general, all articles indicate an increase in the production of reactive oxygen species because of infection. The increase in the production of ROS was also associated with an increase in proteins such as sterile Protein α and TIR containing protein 1 (SARM1) as well as which, changes in the energy substrate were also identified, as a result of the process of infection and viral replication. The change in the energy substrate associated with an increase in the breathing process, and an increase in the MMP, indicate greater availability of ATP for the virus, which may indicate a viral stimulus to obtain energy through changes in cellular homeostasis. This became more evident when it was identified that the morphofunctional changes in the mitochondria occurred close to the CM, an area where viral replication is intense (Keck et al., 2018; Mukherjee et al., 2013).

Regarding the changes in mitochondrial morphodynamics, of the 24 selected articles, only 4 performed analyses related to mitochondrial dynamics, in which, in the consequences, it was identified that arbovirus infections increase the expression of Drp1, indicating a process of fission of mitochondria. In addition, two of the studies showed that the expression of MFN1 and MFN2 decreased with viral infection. In relation to the total number of articles found in the research, few assess the mitochondrial dynamics in infections; of the 24 articles, only 8% show analyses on the mitochondrial dynamics (Chatel-Chaix et al., 2016; Yu et al., 2015).

The mitochondrial elongation was quite expressive in the mitochondria; however, this was not the only change in morphology found; since the articles show that there are partially swollen mitochondria, and inner membrane compaction and mitochondria agglomeration. Therefore, mitochondria respond to viral infections by increasing their respiratory activity and generating morphofunctional changes, however, it was not clear in the studies whether these responses decrease the infectious process or if, due to the increase in the availability of ATP, they facilitate the viral replication process, or even if there were harmful changes to the cell, especially because of the increase in ROS.

Therefore, the increase in total cell ROS was evaluated by some studies, followed by analyses of some biomarkers of oxidative stress. One study stated that there was no increase in the production of reactive species in neutrophils infected by DENV. This result implies that there is no relationship between the increase in ROS and infection of neutrophils by the dengue virus. However, other studies show a relationship between the increase in ROS as a signal for an immune response. The study by Qi Y et al (2015), showed that in infections by DENV, the increase in ROS is associated with the production of IFN-6 and that this affects the cell's MMP (Qi et al., 2015). In addition, another study by Narayanan et al (2011) showed that there is a negative relationship between ROS, SOD, and the increase in TNF- α (Narayanan et al., 2011). Thus, although ROS does not stimulate the production of neutrophils, it is associated with the activation of some mechanisms related to the immune system, indicating that it may be one of the triggers for the activation of the immune response against viral infection. Regarding biomarkers and enzymes, viral infection induces an increase in the activity of SOD and CAT enzymes. In the studies in which SOD levels were reduced, there was an increase in MDA associated with an increase in apoptosis of infected cells. In addition, the levels of GSH and the GSH / GSSG ratio were also reduced in viral infections. In short, the reduction in the activity of the antioxidant defense system is associated with an increase in the MDA biomarker, indicating a possible framework of oxidative

stress during infection. In addition, MDA levels were also associated with increased NO in infected cells and increased apoptosis.

4.2 Study information

The availability of articles that evaluated the damage to mitochondria and the oxidative balance of infected cells is quite scarce when considering the diversity of pathogenic arboviruses that affect humans. Most studies are related to Flavivirus or Togavirus, such as Dengue and Chikungunya; this result may be associated with the importance that these viruses have for public health, principally in this century (WHO, 2014). However, some arboviruses that were also of great importance in this century were not the subject of in vitro studies and research that met the eligibility criteria of this work, for example, none of the studies available with the Zika virus were found in the search.

In the majority of the studies, the cell infection process is not very detailed and, in some articles, important parameters such as infection time, virus MOI, cell concentration, and culture medium details were not identified. These results can serve as a bias for comparing the work, where the evolution of the infectious process may be associated with the concentration of virus used.

In addition, another limitation of the studies was the use of the term “oxidative stress” in studies that evaluated, solely, the production of reactive species or a single biomarker of oxidative stress, therefore, few methodological analyses were performed to indicate that the cells really were experiencing oxidative stress.

5. Final Considerations

The results of this review indicate that in in vitro arbovirus infection models changes in the mitochondrial bioenergetics can help the virus in the replication process, however, cellular changes resulting from the exaggerated increase in reactive species cause an imbalance in the cell REDOX state which can lead to oxidative stress and cell death signaling.

Based on these results, research is suggested to understand how the impacts of ROS caused by arboviruses in in vivo models to understand systemic repercussions in the organism, as well as the role of EROS in the etiology of arboviruses.

Acknowledgments

The authors are grateful to Coordination for the improvement of Higher Education Personnel (CAPES), which provided scholarships for the students and Foundation to support Science and Research from Pernambuco state (FACEPE grant: APQ-0746-2.11/17 - PPSUS 2017 - 10/2017), Brazil.

References

- Azeredo, E. L., Dos Santos, F. B., Barbosa, L. S., Souza, T., Badolato-Corrêa, J., Sánchez-Arcila, J. C., Nunes, P., de-Oliveira-Pinto, L. M., de Filippis, A. M., Dal Fabbro, M., Hoscher Romanholi, I., & Venancio da Cunha, R. (2018). Clinical and Laboratory Profile of Zika and Dengue Infected Patients: Lessons Learned From the Co-circulation of Dengue, Zika and Chikungunya in Brazil. *PLoS currents*, 10, ecurrents.outbreaks.0bf6aeb4d30824de63c4d5d745b217f5. <https://doi.org/10.1371/currents.outbreaks.0bf6aeb4d30824de63c4d5d745b217f5>.
- Banerjee, N., & Mukhopadhyay, S. (2018). Oxidative damage markers and inflammatory cytokines are altered in patients suffering with post-chikungunya persisting polyarthralgia. *Free radical research*, 52(8), 887–895. <https://doi.org/10.1080/10715762.2018.1489131>.
- Beckham, J. D., & Tyler, K. L. (2015). *Arbovirus Infections. Continuum (Minneapolis, Minn.)*, 21(6 Neuroinfectious Disease), 1599–1611. <https://doi.org/10.1212/CON.0000000000000240>.
- Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. *The World Allergy Organization journal*, 5(1), 9–19. <https://doi.org/10.1097/WOX.0b013e3182439613>.
- Camini, F. C., da Silva Caetano, C. C., Almeida, L. T., da Costa Guerra, J. F., de Mello Silva, B., de Queiroz Silva, S., de Magalhães, J. C., & de Brito Magalhães, C. L. (2017). Oxidative stress in Mayaro virus infection. *Virus research*, 236, 1–8. <https://doi.org/10.1016/j.virusres.2017.04.017>.

- Cavalheiro, M. G., Costa, L. S., Campos, H. S., Alves, L. S., Assunção-Miranda, I., & Poian, A. T. (2016). Macrophages as target cells for Mayaro virus infection: involvement of reactive oxygen species in the inflammatory response during virus replication. *Anais da Academia Brasileira de Ciências*, 88(3), 1485–1499. <https://doi.org/10.1590/0001-3765201620150685>.
- Chatel-Chaix, L., Cortese, M., Romero-Brey, I., Bender, S., Neufeldt, C. J., Fischl, W., Scaturro, P., Schieber, N., Schwab, Y., Fischer, B., Ruggieri, A., & Bartenschlager, R. (2016). Dengue Virus Perturbs Mitochondrial Morphodynamics to Dampen Innate Immune Responses. *Cell host & microbe*, 20(3), 342–356. <https://doi.org/10.1016/j.chom.2016.07.008>.
- Cherupanakkal, C., Samadanam, D. M., Muthuraman, K. R., Ramesh, S., Venkatesan, A., Balakrishna Pillai, A. K., & Rajendiran, S. (2018). Lipid peroxidation, DNA damage, and apoptosis in dengue fever. *IUBMB life*, 70(11), 1133–1143. <https://doi.org/10.1002/iub.1925>.
- Datan, E., Roy, S. G., Germain, G., Zali, N., McLean, J. E., Golshan, G., Harbajan, S., Lockshin, R. A., & Zakeri, Z. (2016). Dengue-induced autophagy, virus replication and protection from cell death require ER stress (PERK) pathway activation. *Cell death & disease*, 7(3), e2127. <https://doi.org/10.1038/cddis.2015.409>.
- Dhanwani, R., Khan, M., Bhaskar, A. S., Singh, R., Patro, I. K., Rao, P. V., & Parida, M. M. (2012). Characterization of Chikungunya virus infection in human neuroblastoma SH-SY5Y cells: role of apoptosis in neuronal cell death. *Virus research*, 163(2), 563–572. <https://doi.org/10.1016/j.virusres.2011.12.009>.
- Fernandes-Siqueira LO, Zeidler JD, Sousa BG, Ferreira T, Da Poian AT. Anaplerotic Role of Glucose in the Oxidation of Endogenous Fatty Acids during Dengue Virus Infection. *mSphere*. 2018 Jan 31;3(1):e00458-17. doi: 10.1128/mSphere.00458-17. PMID: 29404419; PMCID: PMC5793041.
- Fontaine, K. A., Sanchez, E. L., Camarda, R., & Lagunoff, M. (2015). Dengue virus induces and requires glycolysis for optimal replication. *Journal of virology*, 89(4), 2358–2366. <https://doi.org/10.1128/JVI.02309-14>.
- Gullberg, R. C., Jordan Steel, J., Moon, S. L., Soltani, E., & Geiss, B. J. (2015). Oxidative stress influences positive strand RNA virus genome synthesis and capping. *Virology*, 475, 219–229. <https://doi.org/10.1016/j.virol.2014.10.037>.
- Keck, F., Brooks-Faulconer, T., Lark, T., Ravishankar, P., Bailey, C., Salvador-Morales, C., & Narayanan, A. (2017). Altered mitochondrial dynamics as a consequence of Venezuelan Equine encephalitis virus infection. *Virulence*, 8(8), 1849–1866. <https://doi.org/10.1080/21505594.2016.1276690>.
- Keck, F., Khan, D., Roberts, B., Agrawal, N., Bhalla, N., & Narayanan, A. (2018). Mitochondrial-Directed Antioxidant Reduces Microglial-Induced Inflammation in Murine In Vitro Model of TC-83 Infection. *Viruses*, 10(11), 606. <https://doi.org/10.3390/v10110606>.
- Kim, S. J., Syed, G. H., Khan, M., Chiu, W. W., Sohail, M. A., Gish, R. G., & Siddiqui, A. (2014). Hepatitis C virus triggers mitochondrial fission and attenuates apoptosis to promote viral persistence. *Proceedings of the National Academy of Sciences of the United States of America*, 111(17), 6413–6418. <https://doi.org/10.1073/pnas.1321141111>.
- Lai, J. H., Wang, M. Y., Huang, C. Y., Wu, C. H., Hung, L. F., Yang, C. Y., Ke, P. Y., Luo, S. F., Liu, S. J., & Ho, L. J. (2018). Infection with the dengue RNA virus activates TLR9 signaling in human dendritic cells. *EMBO reports*, 19(8), e46182. <https://doi.org/10.15252/embr.201846182>.
- Leta, S., Beyene, T. J., De Clercq, E. M., Amenu, K., Kraemer, M., & Revie, C. W. (2018). Global risk mapping for major diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases*, 67, 25–35. <https://doi.org/10.1016/j.ijid.2017.11.026>.
- Lopes, Nayara, Nozawa, Carlos, & Linhares, Rosa Elisa Carvalho. (2014). Características gerais e epidemiologia dos arbovírus emergentes no Brasil. *Revista Pan-Amazônica de Saúde*, 5(3), 55-64. <https://dx.doi.org/10.5123/s2176-62232014000300007>.
- Maynard, N. D., Gutschow, M. V., Birch, E. W., & Covert, M. W. (2010). The virus as metabolic engineer. *Biotechnology journal*, 5(7), 686–694. <https://doi.org/10.1002/biot.201000080>.
- Moher D, Liberati A, Tetzlaff J, Altman DG (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol*. 62(10):1006-12. <https://doi.org/10.1016/j.jclinepi.2009.06.005>.
- Moreno-Altamirano, M. M., Rodríguez-Espinosa, O., Rojas-Espinosa, O., Pliego-Rivero, B., & Sánchez-García, F. J. (2015). Dengue Virus Serotype-2 Interferes with the Formation of Neutrophil Extracellular Traps. *Intervirology*, 58(4), 250–259. <https://doi.org/10.1159/000440723>.
- Mukherjee, P., Woods, T. A., Moore, R. A., & Peterson, K. E. (2013). Activation of the innate signaling molecule MAVS by bunyavirus infection upregulates the adaptor protein SARM1, leading to neuronal death. *Immunity*, 38(4), 705–716. <https://doi.org/10.1016/j.immuni.2013.02.013>.
- Narayanan, A., Amaya, M., Voss, K., Chung, M., Benedict, A., Sampey, G., Kehn-Hall, K., Luchini, A., Liotta, L., Bailey, C., Kumar, A., Bavari, S., Hakami, R. M., & Kashanchi, F. (2014). Reactive oxygen species activate NFκB (p65) and p53 and induce apoptosis in RVFV infected liver cells. *Virology*, 449, 270–286. <https://doi.org/10.1016/j.virol.2013.11.023>.
- Narayanan, A., Popova, T., Turell, M., Kidd, J., Chertow, J., Popov, S. G., Bailey, C., Kashanchi, F., & Kehn-Hall, K. (2011). Alteration in superoxide dismutase 1 causes oxidative stress and p38 MAPK activation following RVFV infection. *PLoS one*, 6(5), e20354. <https://doi.org/10.1371/journal.pone.0020354>.
- Olagnier, D., Peri, S., Steel, C., van Montfoort, N., Chiang, C., Beljanski, V., Slifker, M., He, Z., Nichols, C. N., Lin, R., Balachandran, S., & Hiscott, J. (2014). Cellular oxidative stress response controls the antiviral and apoptotic programs in dengue virus-infected dendritic cells. *PLoS pathogens*, 10(12), e1004566. <https://doi.org/10.1371/journal.ppat.1004566>.
- Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative medicine and cellular longevity*, 2017, 8416763. <https://doi.org/10.1155/2017/8416763>.
- Powers A. M. (2017). Vaccine and Therapeutic Options To Control Chikungunya Virus. *Clinical microbiology reviews*, 31(1), e00104-16. <https://doi.org/10.1128/CMR.00104-16>.

- Qi, Y., Li, Y., Zhang, Y., Zhang, L., Wang, Z., Zhang, X., Gui, L., & Huang, J. (2015). IFI6 Inhibits Apoptosis via Mitochondrial-Dependent Pathway in Dengue Virus 2 Infected Vascular Endothelial Cells. *PLoS one*, 10(8), e0132743. <https://doi.org/10.1371/journal.pone.0132743>.
- Sheeran FL, Pepe S. Mitochondrial Bioenergetics and Dysfunction in Failing Heart (2017). *Adv Exp Med Biol*.982:65-80. doi: 10.1007/978-3-319-55330-6_4. PMID: 28551782.
- Silva da Costa, L., Pereira da Silva, A. P., Da Poian, A. T., & El-Bacha, T. (2012). Mitochondrial bioenergetic alterations in mouse neuroblastoma cells infected with Sindbis virus: implications to viral replication and neuronal death. *PLoS one*, 7(4), e33871. <https://doi.org/10.1371/journal.pone.0033871>.
- Tait, S. W., & Green, D. R. (2012). Mitochondria and cell signalling. *Journal of cell science*, 125(Pt 4), 807–815. <https://doi.org/10.1242/jcs.099234>.
- Terasaki, K., Won, S., & Makino, S. (2013). The C-terminal region of Rift Valley fever virus NSm protein targets the protein to the mitochondrial outer membrane and exerts antiapoptotic function. *Journal of virology*, 87(1), 676–682. <https://doi.org/10.1128/JVI.02192-12>.
- Tung, W. H., Tsai, H. W., Lee, I. T., Hsieh, H. L., Chen, W. J., Chen, Y. L., & Yang, C. M. (2010). Japanese encephalitis virus induces matrix metalloproteinase-9 in rat brain astrocytes via NF- κ B signalling dependent on MAPKs and reactive oxygen species. *British journal of pharmacology*, 161(7), 1566–1583. <https://doi.org/10.1111/j.1476-5381.2010.00982.x>.
- Valero, N., Mosquera, J., Añez, G., Levy, A., Marcucci, R., & de Mon, M. A. (2013). Differential oxidative stress induced by dengue virus in monocytes from human neonates, adult and elderly individuals. *PLoS one*, 8(9), e73221. <https://doi.org/10.1371/journal.pone.0073221>.
- Verma, A. K., Ghosh, S., Pradhan, S., & Basu, A. (2016). Microglial activation induces neuronal death in Chandipura virus infection. *Scientific reports*, 6, 22544. <https://doi.org/10.1038/srep22544>.
- WORLD HEALTH ORGANIZATION (WHO) A global brief on vector-borne diseases (2014). http://apps.who.int/iris/bitstream/10665/111008/1/WHO_DCO_WHD_2014.1_eng.pdf
- Yang, T. C., Lai, C. C., Shiu, S. L., Chuang, P. H., Tzou, B. C., Lin, Y. Y., Tsai, F. J., & Lin, C. W. (2010). Japanese encephalitis virus down-regulates thioredoxin and induces ROS-mediated ASK1-ERK/p38 MAPK activation in human promonocyte cells. *Microbes and infection*, 12(8-9), 643–651. <https://doi.org/10.1016/j.micinf.2010.04.007>.
- Yu, C. Y., Liang, J. J., Li, J. K., Lee, Y. L., Chang, B. L., Su, C. I., Huang, W. J., Lai, M. M., & Lin, Y. L. (2015). Dengue Virus Impairs Mitochondrial Fusion by Cleaving Mitofusins. *PLoS pathogens*, 11(12), e1005350. <https://doi.org/10.1371/journal.ppat.1005350>